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RESEARCH INSTITUTE, NEW DELHI

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OF THE

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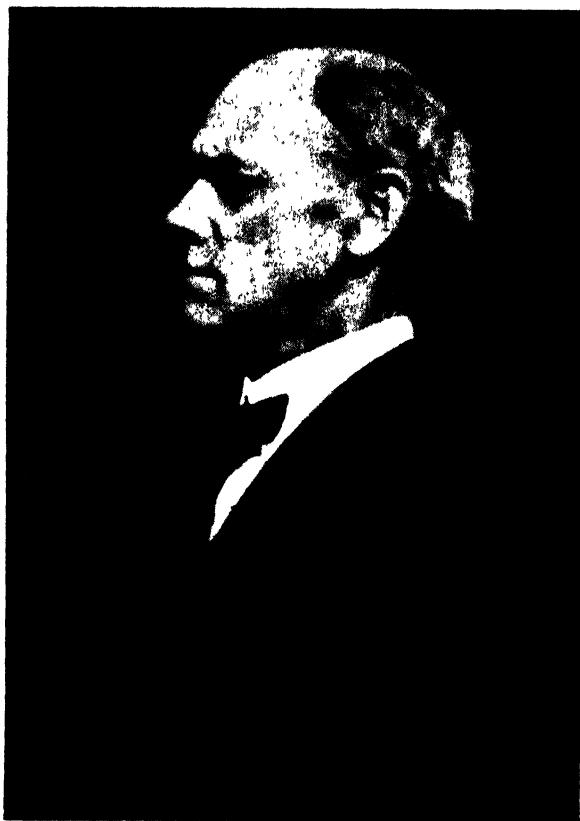
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GEORGE CARL SPENCER, 1870-1912

## GEORGE CARL SPENCER

The death of Dr. George Carl Spencer at his home in Amherst, Massachusetts, on December 16, 1942, marked the passing of an active participator in the work of the Association of Official Agricultural Chemists between 1920 and 1931 as general or associate referee on numerous topics. Although physical disability prevented Spencer from attending any meetings of the Association since 1930, he continued to have a deep interest in the activities of its members. A brief account of his career and work for the Association is, therefore, both fitting and deserving.

Spencer was born at Orange, Massachusetts, on December 18, 1870, and thus at the time of his death lacked only two days of completing his seventy-second year. After obtaining his B.S. degree at Worcester Polytechnic in 1898, he taught chemistry for nine years at the Lowell Textile School. In 1907 he was appointed Assistant Chemist in the U. S. Bureau of Chemistry, but he resigned in 1910 in order to do post-graduate work in organic and agricultural chemistry, physics and botany at Göttingen University. His practical studies at this institution comprised lectures and laboratory work in agricultural chemistry under Tollens, organic chemical research under Wallach, and plant physiological investigations under Berthold. During his stay at Göttingen Spencer was elected "patriarch" of the American Colony, which included among its members B. T. Brooks, W. L. Owen, and other American chemists. Friendships were formed also with H. E. Woodman of Cambridge University and other British co-workers in Wallach's laboratory. After obtaining his Ph.D. at Göttingen in 1913 for a thesis under Wallach on the "Condensation Products of Penta- and Hexacyclic Ketones," Spencer resumed his connection with the Bureau of Chemistry as Organic Chemist in the Drug Division, where in collaboration with W. O. Emery he published articles on the "Estimation of Phenacetin and Salol in Admixture"<sup>1</sup> and on the "Estimation of Theobromine."<sup>2</sup> In 1917-1919, during a temporary transfer to the Bureau of Soils, he performed experiments at the Forest Products Laboratory of the U. S. Department of Agriculture at Madison, Wisconsin, on the distillation and distillates of kelp.<sup>3</sup>

At the meeting of the A. O. A. C. in 1921 Spencer, who had returned to the Bureau of Chemistry as head of the Analytical Reagent Investigations Laboratory, was appointed General Referee on Testing Chemical Reagents. His work on this highly important subject was included in reports which he presented at the meetings of the Association held in 1921,<sup>4</sup> 1922,<sup>5</sup> 1923,<sup>6</sup> 1924,<sup>7</sup> 1925,<sup>8</sup> 1926,<sup>9</sup> and 1927.<sup>10</sup> In the performance of this work, Spencer worked in collaboration with the Analytical Reagents Committee of the American Chemical Society, of which he was a member from 1921 to 1932. His report on "The Testing of Chemicals in the Bureau of Chemistry from 1920 to 1923" was published in Vol. 15 of *Ind. Eng. Chem.* (pp. 1281-2).

At the 1923 meeting of the A. O. A. C. Spencer was appointed an Associate Referee on Cereal Foods. His work on this subject was included in reports on "The Quantitative Determination of Moisture in Wheat Flour"<sup>11</sup> and on "Moisture in Flour and Alimentary Pastes,"<sup>12</sup> and in a series of five reports on "Flour-bleaching Chemi-

<sup>1</sup> *Ind. Eng. Chem.*, **7**, 681-4 (1915).

<sup>2</sup> *Ibid.*, **10**, 605-6 (1918).

<sup>3</sup> *Ibid.*, **12**, 682-4, 786-92 (1920).

<sup>4</sup> *This Journal*, **6**, 1-3 (1922).

<sup>5</sup> *Ibid.*, **6**, 493-7; (1923), **7**, 37-9 (1923).

<sup>6</sup> *Ibid.*, **8**, 106-7 (1924).

<sup>7</sup> *Ibid.*, **8**, 593 (1925).

<sup>8</sup> *Ibid.*, **9**, 347 (1926).

<sup>9</sup> *Ibid.*, **10**, 405 (1927).

<sup>10</sup> *Ibid.*, **11**, 421 (1928).

<sup>11</sup> *Ibid.*, **8**, 301-11, 667-9 (1925), **9**, 404-8 (1926).

<sup>12</sup> *Ibid.*, **10**, 456-8 (1927).



icals.”<sup>13</sup> This same year he was also appointed Associate Referee on Drugs, on which subject he presented reports on “Mercurials”<sup>14</sup> and “Ether.”<sup>15</sup> At the 1928 meeting of the Association Spencer was appointed General Referee on “Metals in Foods,” on which subject he presented reports<sup>16</sup> at the 1929 and 1930 meetings.

In addition to his reports to the Association as General or Associate Referee, he presented in collaboration with O. F. Krumboltz at the 1928 meeting an interesting article on the “Chemical Composition of Alaskan Lichens.”<sup>17</sup> In this contribution there were reported feeding stuff analyses of 21 different varieties of lichens that constitute the principal winter forage for reindeer in Alaska. Another article by V. K. Morton and Spencer on “The Separation of Formic Acid in Food Products by Distillation with Xylene”<sup>18</sup> was presented at the 1925 meeting.

Other articles of which Spencer was the single or collaborative author, appearing in *J. Am. Pharm. Assoc.*, *J. Am. Chem. Soc.*, *J. Oil & Fat Industries*, *J. Wash. Acad. Sci.*, and the Bureau of Chemistry Information Sheets, indicate the wide range of his chemical activities.

Because of a progressively weakening asthmatic condition, provoked by the inhalation of bromine fumes, Spencer was obliged to abandon all chemical work in 1931. He resigned his position as Associate Chemist of the Food Research Division of the Bureau of Chemistry and Soils and spent the remainder of his life in Massachusetts, in the old village of Pelham, Amherst township, where he built a home and where his activities in promoting the social life and welfare of this little community won him a host of friends. Typical of these activities were the Easter Morning sunrise meetings which Spencer held at his home each year on the summit of Pelham Hill and which were attended by hundreds of visitors.

Spencer was a most cultured man. He loved the woods, mountains, and other scenic attractions of his home state, and was fond of the writings of its native authors Emerson and Thoreau. His life was blameless and filled with an ever-present sense of devotion to duty. This was the theme of the remarks made at the funeral exercises in the old church at Pelham on his seventy-second birthday, when a poem that he had written was read. The following verse is quoted therefrom as a farewell message of Spencer's philosophy of life:

“I've lived and loved, I've tried to work,  
To bear my part as best I may;  
Though friends distrust, would fain not shirk  
When Duty pointed out the way.”

C. A. BROWNE

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<sup>13</sup> *This Journal*, 10, 480 (1927); 11, 487-8 (1928); 12, 391-2 (1929); 13, 447-9 (1930); 14, 486-7 (1931).

<sup>14</sup> *Ibid.*, 8, 16-7 (1924); 538-41 (1925); 9, 307-8 (1926)

<sup>15</sup> *Ibid.*, 10, 383 (1927); 11, 360-2 (1928).

<sup>16</sup> *Ibid.*, 13, 416-7 (1930); 14, 434-6 (1931)

<sup>17</sup> *Ibid.*, 12, 317-9 (1929).

<sup>18</sup> *Ibid.*, 9, 221-4 (1926)

## ANNOUNCEMENT

As a result of the war emergency and the congested conditions in the City of Washington, where our meetings have usually been held, the Executive Committee decided not to hold a meeting of the Association in the fall of 1942.

The Committee on Recommendations of Referees circularized all referees and associate referees, seeking reports, so far as available, for publication in *The Journal*, although no opportunity would be afforded this year for the consideration of recommendations and action by the Association on the adoption or revision of methods. It was learned that because of change in work or the press of various war emergency duties, a number of referees would be unable to submit reports this year. Some referees had reports ready for presentation and others expressed the expectancy of forwarding them later. It was decided to publish the reports as they are received, without any attempt to group them under general subjects as has been done in the past. In many instances in this issue there is no general referee report, which means that while there may be a number of associate referee reports on a general subject, they are not complete and may be supplemented. Another change in our customary publication is the elimination of the recommendations, which would now serve no useful purpose. We shall continue to publish contributed papers, and we hope to receive a goodly number during the present year for consideration.

The action of the Executive Committee provides for a continuation of the present officers and committee members and in most cases of the same referees. The few revisions in the list of assignments were made after definite information had been received that a change in duties would not permit the referee or associate referee to continue. This is especially true in regard to referees who have joined the military services. A list of officers, committee members, referees, and associate referees is republished in this number.

HENRY A. LEPPER, *Editor*

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE  
REFEREES OF THE ASSOCIATION OF OFFICIAL  
AGRICULTURAL CHEMISTS FOR THE YEAR  
ENDING NOVEMBER, 1943

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PERMANENT COMMITTEES

*Recommendations of Referees*

(Figures in parentheses refer to year in which appointment expires.)

HENRY A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.),  
*Chairman*

SUBCOMMITTEE A: H. A. HALVORSON (1943) (Department of Agriculture, Dairy and Food, St. Paul, Minn.), *Chairman*; E. L. GRIFFIN (1945), and G. E. GRATTAN (1947).

Enzymes (papain)	Nitrogen
Feeding stuffs	Magnesium and manganese
Sampling	Acid- and base-forming quality
Ash	Calcium and sulfur
Mineral mixed feeds (calcium and iodine)	Copper and zinc
Lactose in mixed feeds	Potash and platinum recovery methods
Fat in fish meal	Insecticides and fungicides
Adulterants of condensed milk products	Total fluorine
Starch	Silicofluoride in admixture with sodium fluoride
Fat in cooked animal feeds containing cereals	Naphthalene in poultry lice products
Crude fat or ether extract	Disinfectants
Filtration aids in crude fiber determination	Leathers and tanning materials
Soluble chlorine	Paints, paint materials, and varnishes
Ammoniacal urea and nitrogen salts	Accelerating testing of paints
Activity of yeast	Varnishes
Fertilizers	Plants
Phosphoric acid (moisture)	Sampling
	Iodine and boron
	Carbohydrates

Zinc  
 Copper and cobalt  
 Chlorophyll, carotene, and iron  
 Hydrocyanic acid  
 Soils and liming materials  
   H-ion concentration of soils of arid  
     and semi-arid regions  
 Boron and fluorine in soils  
 Zinc and copper in soils  
 Exchangeable calcium and magnesium  
 Exchangeable hydrogen in soils  
 Standard solutions  
   Constant boiling hydrochloric acid  
   Sodium thiosulfate solutions

SUBCOMMITTEE B: A. E. PAUL (1943) (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman*; W. F. REINDOLLAR (1945), and H. J. FISHER (1947).

Naval stores  
 Radioactivity  
 Vegetable drugs and their derivatives  
   Chemical methods for ergot alkaloids  
   Theophyllin sodium salicylate  
   Physostigmine in ointments  
   Quinine ethyl carbonate  
   Theobromine and phenobarbital  
   Plasmochin  
   Cinnamyl ephedrine  
   Prostigmine  
   Aminopyrine, ephedrine, and pheno-  
     barbital  
   Strychnine in pills  
   Phenothiazine  
 Synthetic drugs  
   Benzedrine  
   Hydroxyquinoline sulfate  
   Methylene blue  
   Ethyl aminobenzoate  
   Metrazol  
   Bromobarbiturates and thiobarbitu-  
     rates  
   Acetanilid  
   Sulfanilamide derivatives  
   Phenolphthalein and bile salts  
   Atabrine (chinacrin)  
   Sedormid  
   Diethyl stilbestrol  
   Cinchophen and neocinchophen  
 Miscellaneous drugs  
   Microchemical tests for synthetics  
     and alkaloids  
   Mercury compounds (ethanolamine  
     method)  
   Separation of bromides, chlorides, and  
     iodides

Thiocyanate solutions  
 Vitamins  
   Vitamin A  
   Vitamin B<sub>1</sub>  
   Vitamin C  
   Vitamin D—milk  
   Vitamin D—poultry  
   Vitamin K  
   Riboflavin  
   Nicotinic acid  
   Carotene and cryptoxanthin in yellow  
     corn  
   Crude and pure carotene  
 Thyroid  
 Emulsions  
 Compound ointment of benzoic acid  
 Quinine and strychnine  
 Alkali metals  
 Polarograph methods  
 Spectrophotometric methods  
 Preservatives and bacteriostatic agents  
   in ampul solutions  
 Drug bioassays  
   Enteric coatings  
   Posterior pituitary  
   Ergometrine (Ergonovine)  
   Digitalis preparations  
   Glucosides and saponins  
 Cosmetics and coal-tar colors  
   Common ash constituents  
   Alkalies in cuticle removers  
   Arsenic in hair lotions  
   Lead in cosmetics  
   Mercury salts in cosmetics  
   Peroxides in cosmetics  
    $\beta$ -naphthol in hair lotions  
   Pyrogallol in hair dyes  
   Resorcinol in hair lotions  
   Salicylic acid in hair lotions  
   Cosmetic creams  
   Cosmetic powders  
   Dentrifrices and mouth washes  
   Depilatories  
   Hair dyes and rinses  
   Hair straighteners  
   Lip make-up and rouges  
   Lotions for eye and skin  
   Mascara, eyebrow pencils, and eye  
     shadow

Nail cosmetics  
 Ether extract in coal-tar colors  
 Pure dye, impurities, and substrata in pigments  
 Buffers and solvents in titanium tri-chloride titration  
 Halogens in halogenated fluoresceins  
 Intermediates in certified coal-tar colors

Spectrophotometric testing of coal-tar colors  
 Subsidiary dyes in D & C colors  
 Identification of certified coal-tar colors  
 Alizarin and madder lake  
 Urea in deodorants  
 Fill of container methods  
 Deodorants and anti-perspirants

SUBCOMMITTEE C: W. B. WHITE (1943) (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. O. CLARKE (1945), and C. S. LADD (1947).

Canned foods  
   Tomato products  
   Quality factors and fill of container  
   Moisture in dried vegetables  
 Coffee and tea (chlorogenic acid in coffee)  
 Coloring matters in foods  
 Dairy products  
   Neutralizers  
   Decomposition  
   Fat in butter  
   Mold mycelia in butter  
   Lactose in milk  
   Pasteurization of milk and cream  
   Ash in milk and evaporated milk  
   Malting milk (fat)  
   Malting milk (casein)  
   Dried and skim milk (lactic acid)  
   Sugars in sweetened condensed milk  
   Cheese (isolation and identification of fat)  
   Sampling cheese  
   Frozen desserts  
   Moisture  
 Eggs and egg products  
   Unsaponifiable matter and cholesterol  
   Decomposition  
   Added glycerol and salt  
   Dried eggs  
 Fish and other marine products  
   Volatile bases  
   Total solids and ether extract  
   Volatile acids  
   Formic acid in canned salmon and tuna fish  
 Gums in foods  
   Soft curd cheese  
   Mayonnaise and French dressing  
   Frozen desserts  
   Starchy foods  
 Meat and meat products  
   Dried skim milk and soya flour

Metals in foods  
   Selenium  
   Arsenic and antimony  
   Copper  
   Zinc  
   Fluorine  
   Lead  
   Mercury  
   Hydrocyanic acid  
 Microbiological methods  
   Canned fishery products  
   Canned meats  
   Canned vegetables  
   Canned tomatoes and other acid vegetable and fruit products  
   Sugar  
   Eggs and egg products  
   Frozen fruits and vegetables  
   Nuts and nut products  
 Microchemical methods  
 Oils, fats, and waxes  
   Refractometric determination of oil in seeds  
   Unsaponifiable matter  
   Test for peanut oils  
 Preservatives and artificial sweeteners  
   Saccharin in non-alcoholic beverages, semi-solid preparations, and baked goods  
   Esters of benzoic acid  
   Benzoate of soda  
   Sulfur dioxide in meats  
   Monochloroacetic acid  
 Spices and condiments  
   Vinegar  
   Salad dressings  
   Volatile oil in spices (Buchu leaves, fennel seed, and caraway seed)  
   Moisture and ash in spices  
   Ash, salt, and starch in prepared mustard; starch in mustard flour; and volatile oil in mustard seed

SUBCOMMITTEE D: J. W. SALE (1943) (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. A. LeCLERC (1945), and W. C. JONES (1947).

#### Alcoholic beverages

##### Malt

Diastatic activity of malt

Brewing sugars and sirups

Fermentable extracts in brewing sugars and sirups

##### Hops

##### Cereal adjuncts

##### Beer

Color in beer

Iron, copper, and tin in beer

Carbon dioxide in beer

Dextrin in beer

Electrometric method for pH

Sulfur dioxide in beer

##### Wine

Distilled spirits

Spectrophotometric examination of wine and distilled spirits

Formol titrations

Chromatographic absorption of wines

pH in distilled alcoholic beverages

Cordials and liqueurs

#### Cacao products

Lecithin in cacao products

Pectic acid in cacao products

Milk protein in milk chocolate

Chocolate constituents

#### Cereal foods (calcium and iron)

Hydrogen-ion concentration

Starch in raw and cooked cereals

Sugar in bread and other cereal products

Milk solids and butterfat in bread

Rye flour in rye bread and in flour mixtures

Baked products (moisture, ash, protein, fat, and crude fiber)

Fat acidity in grain, flour, corn meal, and whole wheat flour

Baking test for soft wheat flour

Benzoyl peroxide in flour

Carotenoid pigments in flour

Carbon dioxide in self-rising flour

Proteolytic activity of flour

Cellulose in whole wheat flour products

Moisture in self-rising flour and in pancake, waffle, and doughnut flours

Proteins in flour

Phosphated flour

Soya flour

Soya flour in foods (immunological test)

Macaroni products

Noodles and egg-containing products

Flavors and non-alcoholic beverages

$\beta$ -ionone

Lemon oils and extracts

Organic solvents in flavors

Glycerol, vanillin, and coumarin in vanilla and imitation vanillas

Emulsion flavors

Maple flavor concentrates and imitations

Fruits and fruit products

Sodium and chlorides

Polariscopic methods

Titration of acids

Fruit acids

Phosphoric acid ( $P_2O_5$ )

Potassium

(a) Cobaltinitrite procedure

(b) Rapid control method

Cold pack fruits

Sugars and sugar products

Unfermented reducing substances in molasses

Diacetyl

Drying, densimetric, and refractometric methods

Honey and honeydew honey

Sucrose and ash in molasses

Confectionery

Color and turbidity in sugar products

Reducing sugars

Corn sugar and corn sirup

Waters, brine, and salt

Iodides and bromides

Sampling of, and sulfates in, salt

Fluorine and boron

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REFEREES AND ASSOCIATE REFEREES

*Committee A*

ENZYMES (PAPAIN):

*Referee:* R. R. Thompson, Agricultural Experiment Station, Geneva, N. Y.

FEEDING STUFFS:

*Referee:* L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

SAMPLING:

W. L. Hunter, Dept. of Agriculture, Sacramento, Calif.

## ASH:

J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

## MINERAL MIXED FEEDS (CALCIUM AND IODINE):

A. T. Perkins, Kansas State College, Manhattan, Kan.

## LACTOSE IN MIXED FEEDS:

C. W. Sievert, American Dry Milk Inst., Chicago, Ill.

## FAT IN FISH MEAL:

R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

## ADULTERATION OF CONDENSED MILK PRODUCTS:

P. B. Curtis, Agricultural Experiment Station, Lafayette, Ind.

## STARCH:

P. B. Curtis

## FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS:

S. B. Randle, Agricultural Experiment Station, Lexington, Ky.

## CRUDE FAT OR ETHER EXTRACT:

J. J. Taylor, Dept. of Agriculture, Tallahassee, Fla.

## FILTRATION AIDS IN CRUDE FIBER DETERMINATION:

L. S. Walker.

## SOLUBLE CHLORINE:

J. W. Kuzmeski, Agricultural Experiment Station, Amherst, Mass.

## AMMONIACAL UREA AND NITROGEN SALTS:

W. B. Griem, Dept. of Agriculture, Madison, Wis.

## ACTIVITY OF YEAST:

H. J. Witteveen, Dept. of Agriculture, St. Paul, Minn.

## FERTILIZERS:

*Referee.* G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

## PHOSPHORIC ACID (MOISTURE):

W. H. Ross, Bureau of Plant Industry, Washington, D. C.

## NITROGEN:

A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

## MAGNESIUM AND MANGANESE:

J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

## ACID- AND BASE-FORMING QUALITY:

H. R. Allen, Agricultural Experiment Station, Lexington, Ky.

## POTASH AND PLATINUM RECOVERY METHODS:

O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

## CALCIUM AND SULFUR:

Gordon Hart, Dept. of Agriculture, Tallahassee, Fla.



**COPPER AND ZINC:**

W. Y. Gary, Dept. of Agriculture, Tallahassee, Fla.

**INSECTICIDES AND FUNGICIDES:**

*Referee:* J. J. T. Graham, Food Distribution Adm., Washington, D. C.

**TOTAL FLUORINE:**

C. G. Donovan, Food Distribution Adm., Washington, D. C.

**SILICOFLUORIDE IN ADMIXTURE WITH SODIUM FLUORIDE:**

C. G. Donovan.

**NAPHTHALENE IN POULTRY LICE PRODUCTS:**

Rosewell Jinks, Food and Drug Administration, Chicago, Ill.

**DISINFECTANTS:**

*Referee:* C. M. Brewer, Food Distribution Adm., Washington, D. C.

**LEATHERS AND TANNING MATERIALS:**

*Referee:* I. D. Clarke, Bureau of Agricultural Chemistry and Engineering,  
Regional Research Laboratory, Philadelphia, Pa.

**PAINTS, PAINT MATERIALS, AND VARNISHES:**

*Referee:* C. S. Ladd, Office Price Adm., Washington, D. C.

**ACCELERATING TESTING OF PAINTS:**

L. L. Carrick, Agricultural College, Fargo, N. D.

**VARNISHES:**

F. Roberts, Paint and Varnish Laboratory, Bismarck, N. D.

**PLANTS:**

*Referee:* E. J. Miller, Agricultural Experiment Station, East Lansing, Mich.

**SAMPLING:**

E. J. Miller.

**IODINE AND BORON:**

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**CARBOHYDRATES:**

J. T. Sullivan, U. S. Regional Pasture Research Lab., State College, Pa.

**ZINC:**

E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

**COPPER AND COBALT:**

Lillian I. Butler, Agricultural Experiment Station, East Lansing, Mich.

**CHLOROPHYL, CAROTENE AND IRON:**

E. J. Benne.

**HYDROCYANIC ACID:**

To be appointed.

## SOILS AND LIMING MATERIALS:

*Referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

## HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS:

W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

## BORON AND FLUORINE:

J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

## ZINC AND COPPER:

Lewis H. Rogers, Agricultural Experiment Station, Gainesville, Fla.

## EXCHANGEABLE CALCIUM AND MAGNESIUM:

W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

## EXCHANGEABLE HYDROGEN:

W. M. Shaw.

## STANDARD SOLUTIONS:

*Referee:* R. L. Vandaveer, Food and Drug Administration, New Orleans, La.

## CONSTANT BOILING HYDROCHLORIC ACID:

W. H. King, Food and Drug Administration, New Orleans, La.

## SODIUM THIOSULFATE SOLUTIONS:

G. M. Johnson, Food and Drug Administration, St. Louis, Mo.

## THIOCYANATE SOLUTIONS:

E. C. Deal, Food and Drug Administration, New Orleans, La.

## VITAMINS:

*Referee:* E. M. Nelson, Food and Drug Administration, Washington, D. C.

## VITAMIN A:

J. B. Wilkie, Food and Drug Administration, Washington, D. C.

VITAMIN B<sub>1</sub>:

O. L. Kline, Food and Drug Administration, Washington, D. C.

## VITAMIN C:

Otto A. Bessey, Public Health Research Institute, New York City

## VITAMIN D—MILK:

W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

## VITAMIN D—POULTRY:

C. D. Tolle, Food and Drug Administration, Washington, D. C.

## VITAMIN K:

H. J. Almquist, University of California, Berkeley, Calif.

## RIBOFLAVIN:

A. R. Kemmerer, Agricultural Experiment Station, College Station, Tex.

## NICOTINIC ACID:

Harris Isbell, U. S. Public Health Service, Washington, D. C.

CAROTENE AND CRYPTOXANTHIN IN YELLOW CORN:

A. R. Kemmerer.

CRUDE AND PURE CAROTENE:

A. R. Kemmerer.

*Subcommittee B*

NAVAL STORES:

*Referee:* V. E. Grotlich, Food Distribution Adm., Washington, D. C.

RADIOACTIVITY:

*Referee:* To be appointed.

VEGETABLE DRUGS AND THEIR DERIVATIVES:

*Referee:* F. H. Wiley, Food and Drug Administration, Washington, D. C.

CHEMICAL METHODS FOR ERGOT ALKALOIDS:

D. C. Grove, Food and Drug Administration, Washington, D. C.

THEOPHYLLINE SODIUM SALICYLATE:

M. Harris, Food and Drug Administration, Houston, Tex.

PHYSOSTIGMINE IN OINTMENTS:

G. M. Johnson, Food and Drug Administration, St. Louis, Mo.

QUININE ETHYL CARBONATE:

H. G. Underwood, Food and Drug Administration, Chicago, Ill.

THEOBROMINE AND PHENOBARBITAL:

E. C. Deal, Food and Drug Administration, New Orleans, La.

PLASMOCHIN:

F. C. Sinton, Food and Drug Administration, New York City.

CINNAMYL EPHEDRINE:

J. Claggett Jones, 1123 State Office Bldg., Richmond, Va.

PROSTIGMINE:

F. J. McNall, Food and Drug Administration, Cincinnati, Ohio.

AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:

C. D. Wright, Food and Drug Administration, Washington, D. C.

STRYCHNINE IN PILLS:

H. C. Lythgoe, Dept. of Public Health, Boston, Mass.

PHENOTHIAZINE:

V. E. Stewart, Dept. of Agriculture, Tallahassee, Fla.

SYNTHETIC DRUGS:

*Referee:* L. E. Warren, Food and Drug Administration, Washington, D. C.

BENZEDRINE:

J. H. Cannon, Food and Drug Administration, St. Louis, Mo.

**HYDROXYQUINOLINE SULFATE:**

A. M. Allison, Food and Drug Administration, Boston, Mass.

**METHYLENE BLUE:**

H. O. Moraw, Food and Drug Administration, Chicago, Ill.

**ETHYL AMINO BENZOATE:**

E. K. Tucker, State Dept. of Agr. and Industries, Montgomery, Ala.

**METRAZOL:**

S. M. Berman, Food and Drug Administration, Buffalo, N. Y.

**BROMO BARBITURATES AND THIO BARBITURATES:**

L. E. Warren.

**ACETANILID:**

E. H. Wells, Food and Drug Administration, Washington, D. C.

**SULFANILAMIDE DERIVATIVES:**

E. H. Wells.

**PHENOLPHTHALEIN AND BILE SALTS:**

R. Hyatt, Food and Drug Administration, Cincinnati, Ohio.

**ATABRINE (CHINACRIN):**

H. C. Heim, Food and Drug Administration, San Francisco, Calif.

**SEDORMID:**

I. Schurman, Food and Drug Administration, Cincinnati, Ohio.

**DIETHYL STILBESTROL:**

R. D. Stanley, Food and Drug Administration, Chicago, Ill.

**CINCHOPHEN AND NEOCINCHOPHEN:**

R. L. Herd, Food and Drug Administration, Washington, D. C.

**MISCELLANEOUS DRUGS:**

*Referee:* G. K. Glycart, Food and Drug Administration, Chicago, Ill.

**MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS:**

G. L. Keenan, Food and Drug Administration, Washington, D. C.

**MERCURY COMPOUNDS (ETHANOLAMINE METHOD):**

P. S. Jorgensen, Food and Drug Administration, San Francisco, Calif.

**SEPARATION OF BROMIDES, CHLORIDES, AND IODIDES:**

N. E. Freeman, Food and Drug Administration, Atlanta, Ga.

**THYROID:**

M. L. Yakowitz, Food and Drug Administration, San Francisco, Calif.

**EMULSIONS:**

H. F. O'Keefe, Food and Drug Administration, Chicago, Ill.

**COMPOUND OINTMENT OF BENZOIC ACID:**

W. F. Kunke, Food and Drug Administration, Chicago, Ill.

QUININE AND STRYCHNINE:

R. L. Herd, Food and Drug Administration, Washington, D. C.

ALKALI METALS:

W. C. Woodfin, Food and Drug Administration, Atlanta, Ga.

POLAROGRAPH METHODS:

S. Reznak, Food and Drug Administration, Philadelphia, Pa.

SPECTROPHOTOMETRIC METHODS:

J. Carol, Food and Drug Administration, Chicago, Ill.

PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS:

E. M. Beeler, American Pharmaceutical Assoc., Washington, D. C.

DRUG BIOASSAYS:

*Referee:* L. C. Miller, Food and Drug Administration, Washington, D. C.

ENTERIC COATINGS:

H. A. Braun, Food and Drug Administration, Washington, D. C.

POSTERIOR PITUITARY:

R. B. Smith, Food and Drug Administration, Washington, D. C.

ERGOMETRINE (ERGONOVINE):

B. J. Vos, Jr., Food and Drug Administration, Washington, D. C.

DIGITALIS PREPARATIONS:

L. C. Miller.

GLUCOSIDES AND SAPONINS:

L. C. Miller

COSMETICS AND COAL-TAR COLORS:

*Referee:* Dan Dahle, Food and Drug Administration, Washington, D. C.

COMMON ASH CONSTITUENTS:

J. A. Batscha, Food and Drug Administration, New York City.

ALKALIES IN CUTICLE REMOVERS:

R. E. Duggan, Food and Drug Administration, New Orleans, La.

ARSENIC IN HAIR LOTIONS:

H. L. Burrill, Dept. of Health and Welfare, Augusta, Me.

LEAD IN COSMETICS:

W. C. Woodfin, Food and Drug Administration, Atlanta, Ga.

MERCURY SALTS IN COSMETICS:

S. H. Perlmutter, Food and Drug Administration, Minneapolis, Minn.

PEROXIDES IN COSMETICS:

To be appointed.

$\beta$ -NAPHTHOL IN HAIR LOTIONS:

L. C. Weiss, Food and Drug Administration, Los Angeles, Calif.

**PYROGALLOL IN HAIR DYES:**

C. R. Joiner, Food and Drug Administration, St. Louis, Mo.

**RESORCINOL IN HAIR LOTIONS:**

F. M. Garfield, Food and Drug Administration, St. Louis, Mo.

**SALICYLIC ACID IN HAIR LOTIONS:**

H. R. Bond, Food and Drug Administration, Kansas City, Mo.

**COSMETIC CREAMS:**

C. F. Bruening, Food and Drug Administration, Baltimore, Md.

**COSMETIC POWDERS:**

George McClellan, Food and Drug Administration, Baltimore, Md.

**DENTIFRICES AND MOUTH WASHES:**

E. H. Grant, Food and Drug Administration, Boston, Mass.

**DEPILATORIES:**

F. J. McNall, Food and Drug Administration, Cincinnati, Ohio.

**UREA IN DEODORANTS:**

Newell E. Freeman, Food and Drug Administration, Atlanta, Ga.

**HAIR DYES AND RINSES:**

To be appointed.

**HAIR STRAIGHTENERS:**

J. F. Armstrong, Food and Drug Administration, Los Angeles, Calif.

**FILL OF CONTAINER METHODS:**

S. C. Rowe, Food and Drug Administration, Washington, D. C.

**LIP MAKE-UP AND ROUGES:**

E. M. Hoshall, Food and Drug Administration, Baltimore, Md.

**LOTIONS FOR EYE AND SKIN:**

N. E. Foster, Food Chem. Lab., Walter Reed Hospital, Washington, D. C.

**MASCARA, EYEBROW PENCILS, AND EYE SHADOW:**

J. W. Fuller, Dept. of Health and Welfare, Augusta, Me.

**NAIL COSMETICS:**

W. H. Naylor, Food and Drug Administration, Seattle, Wash.

**ETHER EXTRACT IN COAL-TAR COLORS:**

S. S. Forrest, Food and Drug Administration, Washington, D. C.

**PURE DYE, IMPURITIES, AND SUBSTRATA IN PIGMENTS:**

G. R. Clark, Food and Drug Administration, Washington, D. C.

**BUFFERS AND SOLVENTS IN TITANIUM TRICHLORIDE TITRATION:**

O. L. Evenson, Food and Drug Administration, Washington, D. C.

**HALOGENS IN HALOGENATED FLUORESCENTS:**

J. H. Jones, Food and Drug Administration, Washington, D. C.

INTERMEDIATES IN CERTIFIED COAL-TAR COLORS:

K. A. Freeman, Food and Drug Administration, Washington, D. C.

SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:

S. H. Newburger, Food and Drug Administration, Washington, D. C.

SUBSIDIARY DYES IN D&C COLORS:

L. Koch, 537 Columbia St., Brooklyn, N. Y.

IDENTIFICATION OF CERTIFIED COAL-TAR COLORS:

W. F. Whitmore, Polytechnic Inst., Brooklyn, N. Y.

MIXTURES OF COAL-TAR COLORS FOR DRUG AND COSMETIC USE:

W. C. Bainbridge, 537 Columbia St., Brooklyn, N. Y.

DEODORANTS AND ANTI-PERSPIRANTS:

To be appointed.

*Subcommittee C*

CANNED FOODS:

*Referee:* V. B. Bonney, Food and Drug Administration, Washington, D. C.

TOMATO PRODUCTS:

L. M. Beacham, Jr., Food and Drug Administration, Washington, D. C.

QUALITY FACTORS AND FILL OF CONTAINER:

S. C. Oglesby, Food and Drug Administration, Washington, D. C.

MOISTURE IN DRIED VEGETABLES:

J. C. Palmer, Food and Drug Administration, Seattle, Wash.

COFFEE AND TEA (CHLOROGENIC ACID IN COFFEE):

*Referee:* H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

COLORING MATTERS IN FOODS:

*Referee:* C. F. Jablonski, Food and Drug Administration, New York City.

DAIRY PRODUCTS:

*Referee:* G. G. Frary, State Chemical Laboratory, Vermillion, S. D.

NEUTRALIZERS:

F. Hillig, Food and Drug Administration, Washington, D. C.

DECOMPOSITION:

C. S. Myers, Food and Drug Administration, Washington, D. C.

FAT IN BUTTER:

To be appointed.

MOLD MYCELIA IN BUTTER:

J. D. Wildman, Food and Drug Administration, Washington, D. C.

LACTOSE IN MILK:

E. R. Garrison, University of Missouri, Columbia, Mo.

**PASTEURIZATION OF MILK AND CREAM:**

F. W. Gilcreas, Department of Health, Albany, N. Y.

**ASH IN MILK AND EVAPORATED MILK:**

G. G. Frary.

**MALTED MILK (FAT):**

E. W. Coulter, Food and Drug Administration, Chicago, Ill.

**MALTED MILK (CASEIN):**

I. Schurman, Food and Drug Administration, Cincinnati, Ohio.

**DRIED AND SKIM MILK (LACTIC ACID):**

F. Hillig.

**SUGARS IN SWEETENED CONDENSED MILK:**

R. F. Jackson, National Bureau of Standards, Washington, D. C.

**CHEESE (ISOLATION AND IDENTIFICATION OF FAT):**

I. D. Garard, N. J. College for Women, New Brunswick, N. J.

**SAMPLING CHEESE:**

J. B. Snider, Food and Drug Administration, Minneapolis, Minn.

**FROZEN DESSERTS:**

F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

**MOISTURE IN DAIRY PRODUCTS:**

Duncan A. Holaday, Food and Drug Administration, San Francisco, Calif.

**EGGS AND EGG PRODUCTS:**

*Referee:* E. O. Haenni, Food and Drug Administration, Washington, D. C.

**UNSAAPONIFIABLE MATTER AND CHOLESTEROL:**

E. O. Haenni.

**DECOMPOSITION:**

E. O. Haenni.

**ADDED GLYCEROL AND SALT:**

L. C. Mitchell, Food and Drug Administration, Minneapolis, Minn.

**DRIED EGGS:**

K. L. Milstead, Food and Drug Administration, Cincinnati, Ohio.

**FISH AND OTHER MARINE PRODUCTS:**

*Referee:* H. D. Grigsby, Food and Drug Administration, Philadelphia, Pa.

**VOLATILE BASES:**

H. D. Grigsby.

**TOTAL SOLIDS AND ETHER EXTRACT:**

Manuel Tubis, Food and Drug Administration, Philadelphia, Pa.

**VOLATILE ACIDS:**

F. Hillig, Food and Drug Administration, Washington, D. C.



**FORMIC ACID IN CANNED SALMON AND TUNA FISH:**

F. Hillig.

**GUMS IN FOODS:**

*Referee:* F. Leslie Hart, Food and Drug Administration, Los Angeles, Calif.

**SOFT CURD CHEESE:**

M. J. Gnagy, Food and Drug Administration, Los Angeles, Calif.

**MAYONNAISE AND FRENCH DRESSING:**

S. D. Fine, Food and Drug Administration, Cincinnati, Ohio.

**FROZEN DESSERTS:**

F. Leslie Hart.

**STARCHY FOODS:**

M. Elmer Christensen, State Chemist, Salt Lake City, Utah.

**MEAT AND MEAT PRODUCTS:**

*Referee:* R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

**DRIED SKIM MILK AND SOYA FLOUR:**

R. H. Kerr.

**METALS IN FOODS:**

*Referee:* H. J. Wichmann, Food and Drug Administration, Washington, D. C.

**SELENIUM:**

A. K. Klein, Food and Drug Administration, Washington, D. C.

**ARSENIC AND ANTIMONY:**

A. K. Klein.

**COPPER:**

C. A. Greenleaf, National Canners' Association, Washington, D. C.

**ZINC:**

L. V. Taylor, American Can Co., Maywood, Ill.

**FLUORINE:**

P. A. Clifford, Food and Drug Administration, Washington, D. C.

**LEAD:**

P. A. Clifford.

**MERCURY:**

E. P. Laug, Food and Drug Administration, Washington, D. C.

**HYDROCYANIC ACID:**

W. O. Winkler, Food and Drug Administration, Washington, D. C.

**MICROBIOLOGICAL METHODS:**

*Referee:* A. C. Hunter, Food and Drug Administration, Washington, D. C.

**CANNED FISHERY PRODUCTS:**

R. J. Reedy, Fish and Wildlife Service, Washington, D. C.

**CANNED MEATS:**

M. L. Laing, Armour and Company, Chicago, Ill.

**CANNED VEGETABLES:**

E. J. Cameron, National Cannery Association, Washington, D. C.

**CANNED TOMATOES AND OTHER ACID VEGETABLE AND FRUIT PRODUCTS:**

B. A. Linden, Food and Drug Administration, Washington, D. C.

**SUGAR:**

E. J. Cameron.

**EGGS AND EGG PRODUCTS:**

M. T. Bartram, Food and Drug Administration, Washington, D. C.

**FROZEN FRUITS AND VEGETABLES:**

H. E. Goresline, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**NUTS AND NUT PRODUCTS:**

Morris Ostrolenk, Food and Drug Administration, Washington, D. C.

**MICROCHEMICAL METHODS:**

*Referee:* E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

**OILS, FATS, AND WAXES:**

*Referee:* J. Fitelson, Food and Drug Administration, New York City.

**REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:**

Lawrence Zeleny, Food Distribution Adm., Washington, D. C.

**UNSAAPONIFIABLE MATTER:**

Gardner Kirsten, Food and Drug Administration, New York City.

**TEST FOR PEANUT OIL:**

Thomas Riggs, Food and Drug Administration, New York City.

**PRESERVATIVES AND ARTIFICIAL SWEETENERS:**

*Referee:* W. F. Reindollar, Bureau of Chemistry, Department of Health, Baltimore, Md.

**SACCHARIN IN NON-ALCOHOLIC BEVERAGES, SEMI-SOLID PREPARATIONS, AND BAKED GOODS:**

W. F. Reindollar.

**ESTERS OF BENZOIC ACID:**

E. B. Boyce, Department of Public Health, Boston, Mass.

**BENZOATE OF SODA:**

E. B. Boyce.

**SULFUR DIOXIDE IN MEATS:**

C. E. Hynds, State Department of Agriculture and Markets, Albany, N. Y.

**MONOCHLORACETIC ACID:**

J. B. Wilson, Food and Drug Administration, Washington, D. C.

**SPICES AND CONDIMENTS:**

*Referee:* S. Alfend, Food and Drug Administration, St. Louis, Mo.

**VINEGAR:**

A. M. Henry, Food and Drug Administration, Atlanta, Ga.

**SALAD DRESSINGS:**

S. D. Fine, Food and Drug Administration, Cincinnati, Ohio.

**VOLATILE OIL IN SPICES (BUCHU LEAVES, FENNEL SEED, AND CARAWAY SEED):**

J. F. Clevenger, Food and Drug Administration, New York City.

**MOISTURE AND ASH IN SPICES:**

S. Alfend.

**ASH, SALT, AND STARCH IN PREPARED MUSTARD; STARCH IN MUSTARD FLOUR;  
AND VOLATILE OIL IN MUSTARD SEED:**

J. T. Field, Food and Drug Administration, St. Louis, Mo.

*Subcommittee D*

**ALCOHOLIC BEVERAGES:**

*Referee:* J. W. Sale, Food and Drug Administration, Washington, D. C.

**MALT:**

Christian Rask, Albert Schwill Co., Chicago, Ill.

**DIASTATIC ACTIVITY OF MALT:**

Allan D. Dickson, University of Wisconsin, Madison, Wis.

**HOPS:**

Frank Rabak, Bureau of Plant Industry, Washington, D. C.

**CEREAL ADJUNCTS:**

V. E. Munsey, Food and Drug Administration, Washington, D. C.

**BREWING SUGARS AND SIRUPS:**

Stephen Laufer, Schwarz Laboratories, Inc., New York City.

**FERMENTABLE EXTRACTS IN BREWING SUGARS AND SIRUPS:**

P. P. Gray, Wallerstein Laboratories, New York City.

**BEER:**

H. W. Rohde, Schlitz Brewing Co., Milwaukee, Wis.

**DEXTRIN IN BEER:**

H. W. Rohde.

**ELECTROMETRIC METHOD FOR pH:**

Kurt Becker, Siebel Institute, Chicago, Ill.

**SULFUR DIOXIDE IN BEER:**

L. V. Taylor, American Can Company, Maywood, Ill.

**IRON, COPPER, AND TIN IN BEER:**

L. E. Clifcorn, Continental Can Co., Chicago, Ill.

**COLOR IN BEER:**

B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.

**CARBON DIOXIDE IN BEER:**

F. C. Baselt, American Can Co., New York City.

**DISTILLED SPIRITS:**

Peter Valaer, Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.

**SPECTROPHOTOMETRIC EXAMINATION OF WINES AND DISTILLED SPIRITS:**

G. F. Beyer, Bureau of Internal Revenue, Washington, D. C.

**FORMOL TITRATIONS:**

G. F. Beyer.

**CHROMATOGRAPHIC ABSORPTION OF WINES:**

G. K. Hamill, Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.

**pH IN DISTILLED ALCOHOLIC BEVERAGES:**

Maurice Rosenblatt, Schenley Research Inst., Inc., Lawrenceburg, Ind.

**WINE:**

M. A. Amerine, University of California, Berkeley, Calif.

**CORDIALS AND LIQUEURS:**

J. B. Wilson, Food and Drug Administration, Washington, D. C.

**CACAO PRODUCTS:**

*Referee:* W. O. Winkler, Food and Drug Administration, Washington, D. C.

**LECITHIN IN CACAO PRODUCTS:**

J. H. Bornmann, Food and Drug Administration, Chicago, Ill.

**PECTIC ACID IN CACAO PRODUCTS:**

W. O. Winkler.

**MILK PROTEIN IN MILK CHOCOLATE:**

M. L. Offutt, Food and Drug Administration, New York City.

**CHOCOLATE CONSTITUENTS:**

W. O. Winkler.

**CEREAL FOODS (CALCIUM AND IRON):**

*Referee:* V. E. Munsey, Food and Drug Administration, Washington, D. C.

**RYE FLOUR IN RYE BREAD AND IN FLOUR MIXTURES:**

C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.

**MACARONI PRODUCTS:**

R. H. Harris, Agricultural Experiment Station, Fargo, N. D.

**H-ION CONCENTRATION:**

F. A. Collatz, General Mills, Inc., Minneapolis, Minn.

**STARCH IN RAW AND COOKED CEREALS:**

M. P. Etheredge, Miss. State Chemical Lab., State College, Miss.

**FAT ACIDITY GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR:**

Lawrence Zeleny, Food Distribution Adm., Washington, D. C.

**SUGAR IN BREAD AND OTHER CEREAL FOODS:**

R. M. Sanstedt, Agricultural Experiment Station, Lincoln, Neb.

**BAKING TEST FOR SOFT WHEAT FLOUR:**

Robert S. Bailey, National Milling Co., Toledo, Ohio.

**BENZOYL PEROXIDE IN FLOUR:**

Dorothy B. Scott, Food and Drug Administration, New York City.

**CARBON DIOXIDE IN SELF-RISING FLOUR:**

R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.

**MILK SOLIDS AND BUTTERFAT IN BREAD:**

V. E. Munsey.

**PROTEOLYTIC ACTIVITY OF FLOUR:**

Quick Landis, Fleischmann Laboratories, New York City.

**CAROTENOID PIGMENTS IN FLOUR:**

H. K. Parker, Novadel-Agene Corp., Newark, N. J.

**SOYA FLOUR:**

W. L. Taylor, Archer-Daniels-Midland Co., Chicago, Ill.

**SOYA FLOUR IN FOODS (IMMUNOLOGICAL TEST):**

C. S. Ferguson, Department of Public Health, Boston, Mass.

**CELLULOSE IN WHOLE WHEAT FLOUR PRODUCTS:**

Harold Griffith, State Regulatory Laboratory, Bismarck, N. D.

**PHOSPHATED FLOUR:**

Lowell Armstrong, Ballard & Ballard, Louisville, Ky.

**NOODLES AND EGG CONTAINING PRODUCTS:**

E. O. Haenni, Food and Drug Administration, Washington, D. C.

**BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):**

N. H. Walker, National Biscuit Co., New York City.

**MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGHNUT FLOURS:**

L. H. Bailey, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**PROTEINS IN FLOUR:**

D. B. Jones, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

**FLAVORS AND NON-ALCOHOLIC BEVERAGES:**

*Referee:* J. B. Wilson, Food and Drug Administration, Washington, D. C.

 **$\beta$ -IONONE:**

J. B. Wilson.

**LEMON OILS AND EXTRACTS:**

J. B. Wilson.

**ORGANIC SOLVENTS IN FLAVORS:**

R. D. Stanley, Food and Drug Administration, Chicago, Ill.

**GLYCEROL, VANILLIN, AND COUMARIN IN VANILLA AND IMITATION VANILLAS:**

Llewellyn Jones, Food and Drug Administration, Kansas City, Mo.

**EMULSION FLAVORS:**

J. B. Wilson.

**MAPLE FLAVOR CONCENTRATES AND IMITATIONS:**

J. L. Perlman, Department of Agriculture, Albany, N. Y.

**FRUITS AND FRUIT PRODUCTS:**

*Referee:* R. A. Osborn, Food and Drug Administration, Washington, D. C.

**SODIUM AND CHLORIDES:**

R. S. Pruitt, Food and Drug Administration, Cincinnati, Ohio.

**POLARISCOPIC METHODS:**

L. H. McRoberts, Food and Drug Administration, San Francisco, Calif.

**TITRATION OF ACIDS:**

H. M. Bollinger, Food and Drug Administration, San Francisco, Calif.

**FRUIT ACIDS:**

B. G. Hartmann, Food and Drug Administration, Washington, D. C.

**PHOSPHORIC ACID:**

H. Shuman, Food and Drug Administration, Philadelphia, Pa.

**POTASSIUM:**

(a) Cobaltinitrite Procedure: C. A. Wood, Food and Drug Administration, New York City.

(b) Rapid Control Method: H. W. Gerritz, Food and Drug Administration, San Francisco, Calif.

**COLD PACK FRUIT:**

Paul A. Mills, Food and Drug Administration, Seattle, Wash.

**SUGAR AND SUGAR PRODUCTS:**

*Referee:* R. F. Jackson, National Bureau of Standards, Washington, D. C.

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES:

F. W. Zerban, Sugar Trade Laboratory, 113 Pearl St., New York City.

DIACETYL:

J. B. Wilson, Food and Drug Administration, Washington, D. C.

DRYING, DENSIMETRIC, AND REFRACTOMETER METHODS AND REFRACTIVE INDICES  
OF SUGAR SOLUTIONS:

C. F. Snyder, National Bureau of Standards, Washington, D. C.

HONEY AND HONEYDEW HONEY:

G. P. Walton, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

SUCROSE AND ASH IN MOLASSES:

R. A. Osborn, Food and Drug Administration, Washington, D. C.

CONFECTIONERY:

C. A. Wood, Food and Drug Administration, New York City.

REDUCING SUGARS:

R. F. Jackson.

CORN SIRUP AND CORN SUGAR:

G. T. Peckham, Jr., Clinton Co., Clinton, Iowa.

COLOR AND TURBIDITY IN SUGAR PRODUCTS:

J. F. Brewster, National Bureau of Standards, Washington, D. C.

WATERS, BRINE, AND SALT:

*Referee:* A. E. Mix, Food and Drug Administration, Washington, D. C.

FLUORINE AND BORON:

A. E. Mix.

IODIDES AND BROMIDES:

J. T. Tripp, Bureau of Laboratories, Department of Health, Lansing, Mich.

SAMPLING OF, AND SULFATES IN, SALT:

J. T. Tripp.

## REPORT OF SECRETARY-TREASURER

By W. W. SKINNER

For the second time in the 57 years' history of the Association, no annual meeting was held in 1942. As most of the members know, this omission was due to the war emergency and to the congestion in the city of Washington. This condition was evident in 1941, but it grew so much worse in 1942 that the Executive Committee decided that a meeting would entail too many complications. Many invitations were extended to the

Association to hold the meeting in other large cities, but the Committee considered that the added expense would not be justified, in view of the fact that many of the State people would be unable to attend, owing to lack of travel funds and additional defense work in their laboratories.

Since our last meeting there has come to my attention, notice of the death of two former members of the Association, Dr. C. O. Johns and Dr. G. C. Spencer. The former served in the Bureau of Chemistry and was in charge of the protein laboratory. Dr. Spencer also served in the same bureau, working with analytical reagents and metals in foods. He was also in charge of the nitrogen laboratory. Dr. Johns died on April 17, 1942, and Dr. Spencer on December 16, 1942.

Work on the decennial index is proceeding satisfactorily and will be completed early in 1943. The decrease in the amount of copy for *The Journal* in 1942, owing to the postponement of the meeting and to defense work done in many laboratories, will permit the Associate Editor to spend more time on this project. The copy for Volume 25 ran over 1,000 printed pages, the largest volume in the history of the Association.

Postal regulations prohibit the mailing of publications to enemy countries and also to those regions held by the enemy. Many copies of *The Journal* and *Methods of Analysis* have been lost in the attempt to send them to the countries permitted to receive U. S. mail, owing to the loss of shipping vessels. It has also been necessary to have our *Journal* copy passed by the Censor Board. No deletions were ordered until the last number (November) was issued. This number contained reports on drugs that might have been helpful to the enemy.

The sale of the last (1940) edition of *Methods of Analysis* continues at the steady pace that usually follows the first large sales of a new edition. Of the 7,000 copies constituting the last edition, only 1,833 copies remain unsold. These should supply the demands until time for the 1945 revision.

Every effort possible will be made to have a meeting of the Association in 1943. It should be gratifying to the Association that our methods are sufficiently satisfactory to serve as guides in the selection and analysis of food for our armies. These methods are in use in practically all divisions of the armed forces having to do with this most important item.

The affairs of the Association have been running along smoothly, and the meeting postponement and other business matters were taken up by correspondence with the members outside of Washington. The present officers and referees and associate referees will continue in office until the next meeting.

J. W. Bisselle, our former certified accountant, went into service, and the books were audited by Mr. Farr of the firm of Snyder, Farr and Co. The statement, which follows, shows that the financial condition of the Association is very satisfactory.



**STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS  
FOR THE FISCAL YEAR ENDED SEPTEMBER 30, 1942**

**BALANCE, OCTOBER 1, 1941**

Cash on deposit, Lincoln National Bank.....	\$ 6,251.62	
Cash on deposit, Montgomery Mutual Building & Loan Assn.....	1,157.48	\$ 7,409.10

**CASH RECEIPTS**

*Sales:*

Methods of Analysis.....	\$ 7,447.91
Journals.....	5,507.35
Wiley's Principles.....	33.75
Reprints.....	258.95
Advertising.....	550.00

\$13,797.96

Less: Allowances.....	1,571.12	12,226.84
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For books ordered (for others).....	618.27
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From cash in closed banks:

Receiver for Federal-American National Bank & Trust Co.....	\$ 7.31	
Receiver for Commercial National Bank.....	30.27	37.58

Interest on investments (bonds).....	392.50
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Interest on Building & Loan Assn. account.....	46.30
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<b>TOTAL CASH TO BE ACCOUNTED FOR .....</b>	<b>\$20,730.59</b>
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**CASH DISBURSEMENTS**

Salaries.....	\$ 1,478.10
Social security tax.....	28.80
Postage.....	337.40
Association and meeting expense.....	585.25
Auditing.....	150.00
Checks returned.....	43.30
Exchange.....	16.32
Refunds.....	10.00

Printing and binding:

Reprints.....	\$ 757.27	
Journals.....	5,315.66	
Methods of Analysis.....	551.57	6,624.50

Dr. Bailey (present).....	200.00
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Miscellaneous expense.....	117.86
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Books purchased (ordered for others).....	679.47
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U. S. Savings Bonds purchased (par \$7,000).....	5,180.00
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<b>TOTAL CASH DISBURSEMENTS .....</b>	<b>15,451.00</b>
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<b>CASH BALANCE, SEPTEMBER 30, 1942 .....</b>	<b>\$ 5,279.59</b>
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## Represented by:

Cash on deposit, Lincoln National Bank.....	\$ 4,063.21
Cash on deposit, Montgomery Mutual Building & Loan Assn....	1,203.78
Cash on hand.....	12.60
	<u>\$ 5,279.59</u>

**STATEMENT OF INCOME AND EXPENSE  
FOR THE FISCAL YEAR ENDED SEPTEMBER 30, 1942**

**INCOME**

Advertisements.....	\$ 550.00	
Journals.....	5,579.15	
Methods of Analysis.....	7,126.35	
Wiley's Principles.....	41.05	
Reprints.....	263.20	\$13,559.75
	<u>          </u>	
Interest on investments (bonds).....		392.50
Interest on Building & Loan Assn. account.....		46.30
		<u>          </u>
<b>TOTAL INCOME</b> .....		<b>\$13,998.55</b>

**EXPENSE**

Discounts and allowances and refunds.....	\$ 1,581.30	
Printing and binding.....	6,162.03	
Salaries.....	1,492.50	
Postage.....	336.77	
Association and meeting expense.....	337.90	
Social security taxes.....	10.95	
Auditing.....	150.00	
Dr. Bailey (gift).....	200.00	
Exchange.....	16.32	
Cash shortage.....	.04	
Miscellaneous expense.....	135.30	
	<u>          </u>	
<b>TOTAL EXPENSE</b> .....		<b>10,423.11</b>
		<u>          </u>
<b>EXCESS OF INCOME OVER EXPENSES, TO SURPLUS</b> .....		<b>\$ 3,575.44</b>
		<u>          </u>

## REPORT ON LEAD

### DITHIZONE METHOD AND ITS INTERFERENCES

By PAUL A. CLIFFORD (Food and Drug Administration,  
Federal Security Agency), *Associate Referee*

The dithizone reagent of H. Fischer (17) (18) has proved to be an extremely useful tool in the analysis of traces of the heavy metals in a variety of products, notably foods and biological materials. The adaptability of the reagent to this problem as well as the interest chemists have taken in its use is evidenced by the large number of citations in the recent literature. As is well known, under various conditions the reagent forms organo-metallic complexes with many of the heavy metals, and these complexes are usually soluble in ordinary immiscible organic solvents. This makes possible the isolation of certain metals as their dithizone complexes simply by extraction from the aqueous phase with chloroform or carbon tetrachloride. The color changes involved in this extractive separation make the process little short of spectacular.

The dithizone metals and certain properties of their dithizone complexes as given by Fischer (19) and by Fischer and Weyl (20) are listed in Table 1. Certain of these metals, such as indium, manganese, and ferrous iron, yield unstable dithizonates with somewhat variable dithizone "colors"; on the other hand, the solvent colors of many are stable and well defined. In these instances Fischer and Weyl have located the wave-length point of their most prominent absorption bands (Table 1). In the case of at least nine metals in the list, the dithizone reaction has served for their quantitative estimation, and in most of the other instances the reaction serves at least for the qualitative detection of the metal.

When the elements are arranged according to the periodic classification employed by Lundell and Hoffman (28), it is noted that the dithizone metals occupy contiguous positions and form, significantly, a compact block (Figure 1). This block contains all the metals listed by Fischer, and the writer has bounded it so as to include, further, the metals ruthenium, rhodium, osmium, and iridium. While these metals are not mentioned by Fischer, it may be reasoned that they should give at least a partial dithizone reaction when similarities of the Group 8 triads are considered. However, this point has not been checked by the Associate Referee. It would appear certain, at least, that all the possible dithizone metals are enclosed in the block of Figure 1.

### THE LEAD COMPLEX AND DITHIZONE PURITY

According to Fischer (Table 1), divalent lead reacts in alkaline solution with the keto modification of the dithizone reagent to form a complex that

TABLE 1.—*Dithizone metals and properties of their complexes*

METAL	COMPLEX FORM	pH OF REACTION	COLOR IN SOLVENT PHASE (CCl <sub>4</sub> , UNLESS OTHERWISE STATED)	ABSORPTION MAXIMUM (mμ) (CCl <sub>4</sub> SOLUTION)
Cu <sup>++</sup>	keto-Cu (Dz) <sub>2</sub>	0.1 N acid	violet	542
Cu <sup>++</sup>	enol-Cu Dz	alkaline	greenish brown	450
Cu <sup>+</sup>	keto?	acid	red brown	ca. 460
Cu <sup>+</sup>	enol?	alkaline	greenish brown, probably the Cu <sup>++</sup> compound	—
Ag	keto-Ag Dz	acid	yellow	462
Ag	enol-Ag <sub>2</sub> Dz	alkaline	violet (insoluble)	—
Au <sup>+</sup>	keto-AuDz	acid	dirty yellow (partly in- soluble)	indefinite
Au <sup>+</sup>	enol-Au <sub>2</sub> Dz	alkaline	reddish (insoluble)	—
Zn	keto-Zn(Dz) <sub>2</sub>	neutral or weak alkaline	reddish purple	538
Hg <sup>++</sup>	enol-HgDz	weak alkaline	violet	525
Hg <sup>++</sup>	keto-Hg(Dz) <sub>2</sub>	acid	yellow	490
Hg <sup>+</sup>	?	alkaline	weak colors simulating the mercuric com- pounds, probably same due to oxidation	—
Hg <sup>+</sup>	?	acid		—
In <sup>+++</sup>	—	5-6 (narrow range)	red	—
Tl <sup>+</sup>	keto-TlDz	9-12	red	506
Tl <sup>+++</sup>	—	3-4 (incomplete)	gold red	—
Pb <sup>++</sup>	keto-Pb(Dz) <sub>2</sub>	8-10	cherry red	520
Bi	keto-Bi(Dz) <sub>3</sub>	7-9	orange	504
Sn <sup>++</sup>	keto-Sn(Dz) <sub>2</sub>	6-8 (weak alkaline)	purplish red	508
Mn <sup>++</sup>	—	11 (unstable)	brownish red (insoluble) (soluble in CHCl <sub>3</sub> )	—
Fe <sup>++</sup>	—	6-7	violet red	—
Co <sup>++</sup>	keto-Co(Dz) <sub>2</sub>	7-9 (weak alkaline)	violet	ca. 560
Ni <sup>++</sup>	keto-Ni(Dz) <sub>2</sub>	weak alkaline	dirty brown	indefinite
Pd <sup>++</sup>	enol-Pd Dz	weak acid	violet or brownish red flocks, partly soluble in CHCl <sub>3</sub>	—
Pt <sup>++</sup>	—	acid	violet red in aqueous phase—complex insol- uble in CHCl <sub>3</sub> or CCl <sub>4</sub>	—
Cd	keto-Cd(Dz) <sub>2</sub>	strong alkaline (5% NaOH)	red	520

can be represented as Pb(Dz)<sub>2</sub>. Absorption curves of the cherry red solution that the complex gives, both in carbon tetrachloride (20) and chloroform (14), have been described. Liebhafsky and Winslow (27), however, conclude that under alkaline conditions lead and dithizone react mol for mol.

The following experiments were made to check this controversial point. Manipulative details were very simple. Solutions of dithizone in carbon

tetrachloride of about 50 mg./liter strength were saturated with approximately a two-fold excess of lead under the conditions of the colorimetric dithizone determination (3). The cherry red carbon tetrachloride solutions of the complex were carefully filtered and 50 (or 25) ml. portions were analyzed electrolytically for lead (4). Further portions were shaken out with dilute acid, whereby the dithizone equivalent to the lead found was reverted to the uncombined green phase. These solutions were measured photometrically by means of a neutral wedge photometer (13) with an orange (No. 61) filter and 1 cm. cell, and their concentrations were derived by reference to a standard curve for dithizone in purified carbon tetrachloride (10) prepared by measuring dilutions of a dithizone solution made by direct weight from the purified and dried reagent (5). However, when the concentration of the equivalent dithizone was estimated in this manner, ratios of 2.1-2.3 dithizone to 1.0 lead were invariably found. It was concluded that the dithizone used in the gravimetric derivation of the standard reference curve was impure and that dithizone prepared as directed above does not yield a pure product. Hence its photometric curve in carbon tetrachloride is too "flat," whereas the dithizone, freshly liberated from its lead complex with acid, is comparatively pure and yields disproportionately high photometric absorption values.

Apparently some oxidation occurs in this purification process. Fischer (19) states that gentle oxidation of dithizone removes two atoms of hydrogen to form the symmetrical thiocarbodiazone, which has no affinity for metals and produces a yellow color in carbon tetrachloride or chloroform. This yellow product can be reduced back to dithizone with the proper reagents; Fischer and Leopoldi (21) recommend dilute sulfur dioxide water for this purpose and for the preservation of carbon tetrachloride solutions of dithizone. (The efficacy of sulfur dioxide water as a preservative has been checked by the Associate Referee (10) but it was found that it will not preserve a carbon tetrachloride solution of dithizone indefinitely under ordinary laboratory conditions and will not restore a solution that has undergone too drastic oxidation.)

A solution of dithizone in purified carbon tetrachloride of 10.0 mg./liter strength was then prepared and read through a 1.0 cm. cell with filter No. 61. It gave an absorbency value of 1.02. When the stock solution from which it was prepared was allowed to stand overnight in the ice-box under dilute sulfur dioxide water and a similar dilution was made after the solution had regained room temperature, the value for absorbency was increased to 1.10. Because the yellow phase of partially oxidized dithizone does not absorb appreciably in the orange, whereas green dithizone itself absorbs strongly, it may be assumed that this increase was due to regeneration of dithizone from its yellow oxidation products. Accordingly, the slope of the standard dithizone curve was increased by this absorbency ratio, and the assumption was made that corresponding concentration

values would not be altered appreciably if the reversible oxidation-reduction indicated by these data involved only 2 hydrogen atoms. Using this revised standard dithizone curve the Associate Referee repeated the experiment. The sulfur dioxide-treated carbon tetrachloride stock solution was used to prepare the 50 mg./liter dilution, and dilute sulfur dioxide water was used in "stripping" the filtered solution of the lead complex. The resulting carbon tetrachloride solution of dithizone was diluted 1:5 with purified carbon tetrachloride and read in the photometer. Corrected for this dilution, a value of 48.0 mg./liter, or  $1.875 \times 10^{-4}$  mols/liter, was obtained for the concentration of dithizone in the solution of its lead complex. Electrolysis of a 50 ml. portion of this solution gave 0.948 mg. of lead, calculating to  $0.915 \times 10^{-4}$  mols Pb/liter. This ratio is close to 1 Pb:2 Dz (found, 1:2.05) and it would appear that the formula of the complex under these conditions is, in fact,  $\text{Pb}(\text{Dz})_2$ .

Probably pure dithizone has never been obtained in the dry state. Its preparation must certainly involve painstaking purification and careful drying under the proper reducing conditions. Proof of its purity must be established by assay of its nitrogen and sulfur content. The true melting point should be established, and as a further criterion of purity the value of its molecular extinction coefficient at 620  $m\mu$  in carbon tetrachloride solution should be derived. Sulfur dioxide is undoubtedly of great value in purifying and stabilizing dithizone in carbon tetrachloride, but it is not known whether it will entirely reduce a partially oxidized dithizone solution back to its theoretical strength. The concentration of sulfurous acid appears to be critical (10). If absorption of the carbon tetrachloride solution at 620  $m\mu$  is taken as a test of purity, the treated solution should be closely observed until this absorption attains a maximum. At this point, and before it begins to decline, the dithizone solution is probably pure. However, it is seen that the preparation of pure, dry dithizone may be a formidable project, and would entail more work than has been attempted here. The reagent as ordinarily prepared, even if not pure, is entirely serviceable.

The transmission curves and the molar extinction coefficients reported by Liebhafsky and Winslow (27) were checked fairly well when 10 mg./liter solutions of dithizone in carbon tetrachloride were shaken out with dilute sulfur dioxide water and read with the General Electric recording spectrophotometer through a 1 cm. cell. These investigators report molar extinctions at 450, 514, and 620  $m\mu$  as being, respectively, 19000, 4740 and 30400. The Associate Referee found in one case respective values of 18700, 4510, and 31100 and again, 17800, 5400, and 29700.

#### INTERFERENCES

A reagent that will react under various conditions with upwards of 17 metals is certainly not "specific." However, by proper pH control, and

particularly through the use of differential complexing or "masking" ions, the reactions of the dithizone metals can be broken down into groups. In this respect the dithizone reaction differs in no wise from other schemes of chemical analysis, most of which depend upon the reactions of groups. (Probably no reagent is specific for a particular element, and experienced analysts look first to possible interferences in any proposed analytical procedure.) Thus, in alkaline solution and in the presence of excess cyanide ion dithizone will extract lead, bismuth, stannous tin, and thal- lous thallium. As has often been pointed out, the latter three metals constitute the interferences to be guarded against in the dithizone-lead determination. Figure 1 shows that these metals, forming cyanide-stable dithizonates, again fall within a compact group. (Indium is included as one of the "cyanide-fast" metals, but it should cause no interference in the lead procedure as its dithizone reaction is limited to a very narrow, slightly acid range, pH 5-6.)

The spectrograms of Figure 2 illustrate the general transmission characteristics of the lead, tin, thallium, and bismuth dithizonates in carbon tetrachloride solution after adjustment to equal transmission values at 510 m $\mu$ . Certain differences are apparent, especially between the lead and bismuth and the lead and tin complexes. The lead complex, for example, transmits quite freely in the blue while the bismuth dithizonate is relatively opaque. Tin dithizonate transmits blue light quite well, but differs from lead in that its complex shows considerably more absorption in the region of 550-560 m $\mu$ . Should bismuth or tin occur as interferences in the photometric lead method, these differences suggest the possibility of a simple method for their detection.

The usual procedure for the dithizone-lead determination is to estimate the metal from the amount of light absorption at 510 m $\mu$ , where the lead complex absorbs strongly. Absorption at other points in the spectrum heretofore has been disregarded, nevertheless the "mixed colors" in a standard lead series yield definite absorption values at any selected spectral point. It has been the custom of the Associate Referee, when establishing a standard curve for a certain lead range with a given batch of dithizone, to read the standard colors on the neutral-wedge photometer, not only with filter No. 51, but also with auxiliary blue (No. 45) (22) and yellow-green (No. 56) filters, thereby obtaining "normal curves" at these wave lengths (Figure 3, solid lines—filter numbers indicate approximate filter centroids).

*Bismuth.*—Thus a bismuth interference with a resultant value too high for lead is revealed by causing abnormally high absorption values with filter No. 45. The amount of interfering bismuth that can be detected in this manner depends upon the fundamental differences in absorption of the two metal complexes in this wave-length band and upon the reproducibility of the dithizone colors and precision of their measurement. Experi-

ment showed that photometric readings with the above instrument and auxiliary filters are reproducible within at least 1 mm. of scale (about 0.015 density), and this degree of precision was taken to represent the limit of bismuth detection. In other words, if the No. 45 reading was more than 1.0 mm. higher than that normal for the amount of lead indicated by the reading with No. 51, the presence of bismuth was considered to be probable. At the same time the reading with filter No. 56 would be inordinately low. (Scale readings with this instrument are proportional to density, hence inversely related to transmission.) Detection of interfering bismuth in quantity equal to about 5 per cent of the top of the lead range employed, e.g., 1 mmg. of bismuth in the 0-20 mmg. lead range, is possible.

It was found that standard dithizone solutions in chloroform deteriorated upon standing, with the gradual appearance of a yellow phase that absorbed in the blue and vitiated the standard No. 45 curve. The difficulty was overcome when it was found that standard carbon tetrachloride solutions of dithizone could be stabilized with the aid of sulfur dioxide water and storage in the cold; reference curves with filter No. 45 were unchanged over periods of months (10). It was found necessary to increase the concentration of carbon tetrachloride-dithizone standard solutions to about three times that specified for chloroform for a given lead range because of the lesser solubility of the lead complex in the tetrachloride. (Possible advantages of the use of carbon tetrachloride in the photometric mixed color procedure have been discussed (11). It is also cheaper than chloroform and less volatile, and its lesser solubility in water makes unnecessary previous saturation with tetrachloride of the 1 per cent nitric acid used in developing the standard colors of a lead range.) The use of hydroxylamine and freshly purified dithizone in the preliminary extraction was also effective in securing stable dithizone colors, especially when read with the auxiliary blue filter. While standard dithizone solutions used in the final color development were made up in tetrachloride, chloroform solutions of dithizone were still employed for the preliminary extraction because of their greater efficacy. However, the 50 ml. of 1 per cent stripping acid was washed with 10 ml. of clear tetrachloride to remove dissolved chloroform before the color development.

A typical set of standard curves for bismuth is contrasted with the curves for lead in Figure 3. The colors for both metals were developed under identical conditions with the same dithizone solution. The dithizone colors for bismuth are stable, and the metal can be determined in the same manner as is lead (23). This fact, as well as the considerable difference between scale readings with filters 45 and 56 for equivalent amounts of the two metals, makes possible not only the detection of interfering bismuth in the lead method, but also its co-determination with lead.

Two sets of data collected in October, 1938, are presented in illustration. In the first case, 10 solutions of pure lead and bismuth were measured



into beakers (amounts unknown to the writer). These were rinsed into separatory funnels, and the mixed metals were given a preliminary extraction with dithizone after the addition of citrate, cyanide, and ammonia. The extracts were then stripped with 50 ml. of 1 per cent nitric acid as specified in the method, and the mixed colors were developed with the same standard carbon tetrachloride solution of dithizone as that used in obtaining the standard curves of Figure 3. The use of filter 45 in the detection of bismuth is illustrated by the following filter readings on the 10 unknowns (Table 2).

The data of Figure 3 make possible the co-determination of lead and bismuth. Because the amounts of either pure bismuth or lead indicated

TABLE 2.—Use of filter 45 in the detection of bismuth—pure solutions

NO.	FILTER 45	FILTER 51	FILTER 56	"Pb" FROM 51	NORMAL 45 FOR THIS AMT. Pb	Bi INDICATED?	ADDED	
				mmg.			Pb	Bi
1	15.7	23.2	13.0	3.8	14.0	Yes	2.0	1.0
2	41.8	96.3	31.7	18.5	29.7	Yes	10.0	8.0
3	29.8	67.1	25.3	12.6	23.4	Yes	8.0	4.0
4	11.4	14.1	10.3	1.9	12.0	No	1.0	—
5	15.0	29.0	15.2	4.9	15.1	No	4.0	—
6	36.9	86.0	29.7	16.4	27.4	Yes	10.0	6.0
7	15.7	24.2	12.8	4.0	14.1	Yes	2.0	1.0
8	34.1	95.7	33.6	18.4	29.7	Yes	14.0	4.0
9	21.8	47.0	20.0	8.6	19.1	Yes	6.0	2.0
10	13.0	19.2	12.5	3.0	13.1	No	2.0	—

Blank of determination as Pb = 0.8 mmg.

by the 51 reading are almost equivalent, the amount of bismuth in a color mixture can be estimated quite closely by a simple interpolation of the 45 reading. Thus if the 51 reading is taken to represent all lead, a normal 45 reading for this amount can be called X. If the 51 reading is taken to represent all bismuth, a normal 45 reading for this amount of bismuth could be called Y. Then, calling the actual 45 reading on a mixture, A, the proportion of bismuth is about  $A-X/Y-X$ .

A neater and more nearly exact method of resolving the lead and bismuth mixed colors is by a set of simultaneous equations, as outlined for a two-component system by Knudsen et al (26). The essential data, represented in the graphs of Figure 3, consist in the derivation of the slopes of the curves with two filters relating scale to quantity of metal for both pure lead and bismuth. Scale reading in millimeters, instead of density, can be used, because with this instrument the two are directly related. The method of least squares can be employed in evaluation of these slopes according to the procedure outlined in *Methods of Analysis* (6), except that the reciprocal value of the slope so found, i.e., density (or mm. scale)

<u>THE DITHIZONE METALS</u>																	
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	A
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Cb	Mo	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	Rn	
	Ra	Ac	Th	Pa	U												

FIG 1

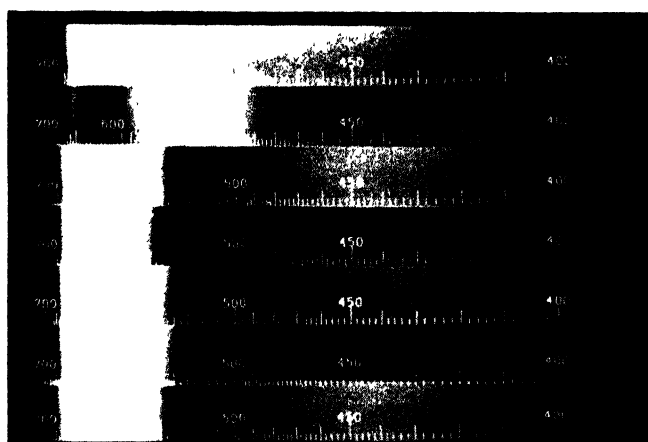


FIG. 2 SPECTROGRAMS OF VARIOUS DITHIZONE METALS.

Top to bottom --1. Light source through cell and solvent. 2 Dithizone in carbon tetrachloride, 6 mg per liter. 3 Lead dithizone complex 4 Tin dithizone complex 5. Thallium dithizone complex. 6 Bis-muth dithizone complex 7. Lead dithizone complex.

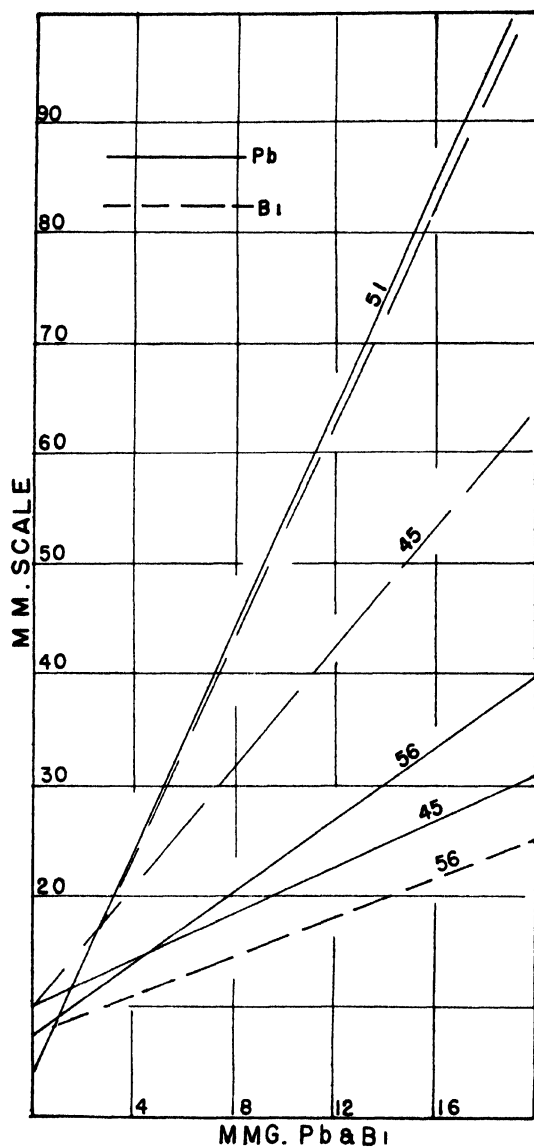


FIG. 3.—STANDARD CURVES FOR LEAD AND BISMUTH WITH NEUTRAL WEDGE PHOTOMETER.

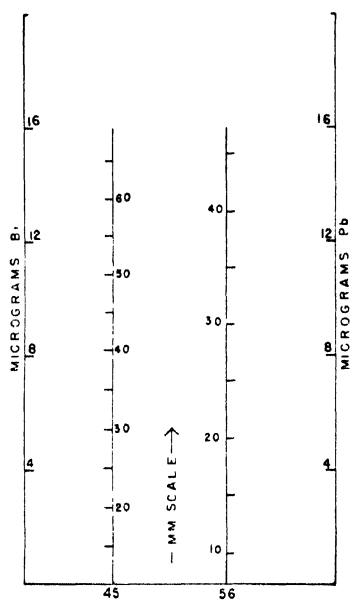


FIG. 4 —NOMOGRAPH FOR RESOLVING DITHIZONE MIXTURES OF LEAD AND BISMUTH.

per mmg. lead or bismuth, should be used. Because these calculations presuppose that all curves start at the origin, the average value of the intercepts for both metals with each filter on the vertical (mm.) axis must be subtracted from the corresponding filter readings on unknown mixtures. (Theoretically, these intercept values should be identical for a particular filter, as both curves should begin at the same point for zero metal, but actually the two values may calculate out with a few tenths mm. difference.) Then:

$$C_1 = \frac{K_2^a D^b - K_2^b D^a}{K_2^a K_1^b - K_2^b K_1^a}, \text{ and } C_2 = \frac{K_1^b D^a - K_1^a D^b}{K_2^a K_1^b - K_2^b K_1^a},$$

where  $C_1$  = mmg. Pb,  $C_2$  = mmg. Bi,  $K_1^a$  and  $K_1^b$  = the slopes of the lead curves expressed as above with two filters a and b;  $K_2^a$  and  $K_2^b$  the slopes

TABLE 3.—*Co-determination of lead and bismuth from data with two filters*

	METAL (MMG.)	DETERMINATION (MMG.)									
		1	2	3	4	5	6	7	8	9	10
Filters 56 and 45	Lead (calculated)	2.8	11.1	8.8	2.5	5.2	10.7	3.2	15.5	7.0	3.2
	Lead (-blank)	2.0	10.3	8.0	1.7	4.4	9.9	2.4	14.7	6.2	2.4
	Bismuth (calculated)	1.0	7.5	3.9	None	None	5.8	.9	2.8	1.6	None
Filters 56 and 45	Lead (calculated)	3.0	11.0	9.1	1.9	4.9	10.7	2.8	14.6	7.0	3.3
	Lead (-blank)	2.2	10.2	8.3	1.1	4.1	9.9	2.0	13.8	6.2	2.5
	Bismuth (calculated)	1.0	7.5	3.8	None	None	5.8	1.0	3.2	1.6	None
Added	Lead	2.0	10.0	8.0	1.0	4.0	10.0	2.0	14.0	6.0	2.0
	Bismuth	1.0	8.0	4.0	—	—	6.0	1.0	4.0	2.0	—

Blank of determination as Pb = 0.8 mmg.

of the bismuth curves for the same filters, and  $D^a$  and  $D^b$  the scale readings of the unknown, corrected for intercept, with filters a and b. L. F. Knudsen (31) points out that it is unnecessary to make this correction if values for the intercepts are included in the two above expressions, which thereby take slightly different forms.

In applying these formulas to the 10 unknowns, about any combination of two of the three filters can be used. Thus if filter 51 is called a and 45 called b;  $K_1^a = 4.976$  and  $K_1^b = 1.058$ ;  $K_2^a = 4.875$  and  $K_2^b = 2.674$ ; intercepts for filters a and b are respectively 4.0 and 10.0 mm. Results calculated from the data with filters 56 (a) and 45 (b) were slightly closer to the true values; ( $K_1^a = 1.598$  and  $K_2^a = .884$ ; intercept for 56 = 7.4 mm.). Calculated values with two combinations of filters are compared with added amounts of lead and bismuth in Table 3.

The calculations prove, incidentally, that the blank of the determination is actually lead, since calculated results do not check well with added

amounts of lead until the experimentally determined blank of 0.8 mmg. is subtracted. It is unnecessary to correct the calculated bismuth values for apparently there is no perceptible bismuth blank in most analytical work. This checks the observations of Hubbard (23). Where bismuth is reported as "none," zero or small negative values were yielded by the calculations.

R. U. Bonnar (31) has employed the method of simultaneous equations in resolving mixtures of dyes and has pointed out short cuts in the mathematical method.

The second set of data was obtained upon 10 ml. portions of beef blood to which had been added unknown quantities of lead and bismuth. In this case only a one-half aliquot of the 1 per cent nitric acid in which the metals were finally isolated was taken for the colorimetric determination

TABLE 4.—Use of filter 45 in detection of bismuth—blood unknowns

NO.	FILTER 45	FILTER 51	FILTER 56	"Pb" FROM 51	NORMAL 45 READING FOR THIS AMT. Pb	BI INDICATED?	ADDED	
							Pb	Bi
				(mmg.)			(mmg.)	
1	19.3	46.3	19.2	4.5	17.0	Yes	2.0	2.0
2	19.2	55.0	22.4	5.4	18.7	No	6.0	—
3	37.3	112.5	38.0	11.5	30.4	Yes	15.0	6.0
4	26.4	91.0	33.8	9.2	26.0	No	15.0	—
5	28.4	76.0	27.7	7.6	22.9	Yes	8.0	4.0
6	34.0	92.8	32.7	9.4	26.4	Yes	10.0	5.0
7	27.0	86.2	32.5	8.7	25.0	Yes	12.0	1.0
8	22.9	57.0	22.0	5.6	19.1	Yes	4.0	3.0
9	34.4	106.5	37.0	10.8	29.0	Yes	14.0	4.0
10	19.2	52.5	22.0	5.1	18.2	?	5.0	0.5

and measured with a 0–10 mmg. lead range. Curves and data from which the calculations were made are not shown, but they are similar to those employed with the series of pure solutions. The total blank of the determination, including reagent blank and the naturally occurring lead content of the blood itself, was 5.0 mmg. The use of filter 45 in the detection of bismuth interference is illustrated in Table 4.

It is noted that readings with auxiliary filter 45 detected the presence of bismuth except in the case of No. 10, where an interference was doubtful. Resolution of the lead and bismuth mixtures by the method of simultaneous equations outlined above yielded the results shown in Table 5 for the same filter combinations.

Apparently calculated values for bismuth of below 0.5 mmg. are of little significance. One mmg. is readily detectable and, as in Table 4, the presence of bismuth in No. 10 would be suspected but not proven. The blank of the determination is again shown to be actually lead.

It may be well to point out here that the complete dithizone extraction of the larger quantities of bismuth from a mixture of extraneous salts, notably citrate, is more difficult than is the alkaline extraction of lead. This fact should be noted by investigators interested in the above co-determination of the two metals. Hubbard (23) resorts to a sulfide method of preliminary isolation of bismuth from his samples. The Associate Referee has noted that under the conditions of the final color development for lead, and depending upon the strength of the standard dithizone solution used, considerable bismuth may remain in the aqueous phase. However, under set conditions, this partition appears to be constant, and the standard curves for bismuth of Figure 3 were as reproducible as were those for lead.

The method of testing colorimetric extracts for the presence of bismuth

TABLE 5.—*Co-determination of lead and bismuth in blood samples*

	METAL (MMG.)	1	2	3	4	5	6	7	8	9	10
Filters 51 and 45	Lead (calculated)	7.6	10.5	18.9	18.2	12.1	14.3	16.3	9.0	18.6	9.7
	Lead (-blank)	2.6	5.5	13.9	13.2	7.1	9.3	11.3	4.0	13.6	4.7
	Bismuth (calculated)	1.6	0.3	4.9	0.3	3.8	5.3	1.4	2.6	3.7	0.6
Filters 56 and 45	Lead (calculated)	7.7	10.4	18.8	18.2	12.4	15.2	16.9	9.1	18.7	10.1
	Lead (-blank)	2.7	5.4	13.8	13.2	7.4	10.2	11.9	4.1	13.7	5.1
	Bismuth (calculated)	1.5	0.3	4.9	0.3	3.6	4.9	1.1	2.5	3.6	0.5
Added	Lead	2.0	6.0	15.0	15.0	8.0	10.0	12.0	4.0	14.0	5.0
	Bismuth	2.0	—	6.0	—	4.0	5.0	1.0	3.0	4.0	0.5

Blank of determination as lead = 5.0 mmg.

by means of readings with auxiliary filter 45 has been employed to test the efficacy of the dithizone separation of lead from bismuth as proposed by Willoughby et al (30). Thus 100 mmg. of bismuth could be separated cleanly from 15 mmg. of lead at pH 2.0 with only two extractions with Willoughby's dithizone reagent; with 500 mmg. of bismuth and two extractions, traces of bismuth still remained in the extracted solution. However, three extractions, as recommended by these authors, effectively removed all trace of bismuth, with no concurrent loss of lead.

Photometric data with two filters are readily applied to a nomographic resolution of the lead and bismuth mixed colors, and the data of Figure 3 with filters 45 and 56 are used in the construction of such a nomograph (Figure 4). It is readily seen how this method is applied to the data recorded in Table 2.

*Tin.*—Tin has been found not to be a serious interference in the dithizone procedure because it is normally oxidized to the stannic form during the sample preparation, and in this valence it does not react with dithizone. Possibly it has been too much stressed as an interference in the dithizone lead method. Tin is present in large quantities in canned foods,

but it seldom causes trouble. In using the dithizone procedure for the determination of lead in various products packaged in tin or tin-lined collapsible tubes, the writer has found no interference due to tin. Stannic tin is not reduced with hydroxylamine under the conditions of the alkaline dithizone extraction.

If it should occur as interference in the lead method stannous tin reveals itself by causing abnormally high density values (high scale readings) with filter 56 (Figure 2), and this is the best spectral point for its detection. Experiments similar to those with bismuth show that a tin contamination of the mixed color equal to about 10 per cent of the top of the lead range employed, e.g., 2 mmg. for the 0-20 mmg. range, can be detected if a reading 1 mm. above "normal" for indicated lead is taken as the limit of detection. It was found, however, that upwards of 50-100 milligrams of tin must be present in the preliminary extraction before 1-2 microgram quantities are extracted with dithizone and revealed in the final color development. Readings in the blue with filter 45 are not much affected; thus while tin and bismuth might conceivably neutralize one another with filter 56 if both are present as interferences in a color mixture, filter 45 would still detect bismuth and thus indicate the presence of both.

*Thallium.*—The remaining interference in the lead method is that of thallium. The great similarity of its dithizone color to that of lead (Figure 2) would probably render inadequate the differential photometric methods described here. However, the rarity of thallium makes such interference extremely unlikely; to the writer's knowledge it has never been encountered as interference by any of the numerous investigators that have applied the dithizone lead method to a variety of products. Should there develop a need for its separation in the microdetermination of lead several methods could be adapted, of which the simplest is probably a dithizone separation at pH 6.0-6.4. The procedure is similar in principle to the separation of lead and bismuth at pH 2.0, except in this case the lead is extracted and thallium remains in the aqueous phase. Thus the writer has separated cleanly 50 mmg. of lead from 1 mg. of thallium by extracting the lead from a mixture of the pure metals with three 20 ml. portions of dithizone in chloroform of 100 mg./liter strength. This separation is another instance of how properly chosen conditions of pH and dithizone strength can add to the specificity of the dithizone reaction.

*Indium.*—The presence of indium in ordinary work has been ignored, but should it be found as an interference in the lead method (which is highly improbable), methods for its separation could no doubt be developed.

#### DITHIZONE METHOD APPLIED TO URINE

In the minds of certain chemists the apparent lack of specificity of the dithizone reaction has promoted a distrust of its use in the analysis for a

particular metal. Fairhall and Keenan (16) contend that the colorimetric dithizone method for lead yields results too high by several hundred per cent when they apply it to the analysis of lead in urine, and compare results with those obtained by a chromate-titrimetric procedure. Their dithizone values were obtained upon 100 ml. portions of urine and their chromate values by separating the lead from the total remainder, working up, in some cases, over 100 liters. The investigation of such large volumes of urine was admittedly laborious and time-consuming, but these authors thought that the advantage gained was the securing of enough lead for objective examination and identification. These chromate values were taken as correct, and the discrepancy between them and the higher dithizone values is regarded as due to the extraction of interfering metals, which Fairhall and Keenan do not directly identify. They note, however, that other investigators (27) have reported high values for lead with the dithizone method in the presence of copper, and suggest that yellow oxidation products of the dithizone reagent may simulate the lead color and cause high results. Fairhall has also noted (15) that still another investigator has reported zinc to be an interference in the dithizone method for lead.

The conclusions of Fairhall and Keenan disparage the general reliability of the dithizone-lead method, which is utilized quite extensively by the Association in its official and tentative methods for lead. For this reason a considerable amount of work was done on urine by the Associate Referee to show that the dithizone method, properly applied, is both accurate and specific, and that the results it yields on small (100 ml.) portions of urine correlate well with those of other methods applied to much larger quantities.

About 17 liters of mixed urine was collected in a glass carboy after the addition to the carboy of 25 ml. of redistilled chloroform as a preservative. Collection took two days, and during the second day 25 ml. of redistilled nitric acid was mixed in to minimize formation of a phosphate sediment. This was at the suggestion of E. P. Laug (31), who has found large volumes of urine difficult to sample unless slightly acidified. When filled, the carboy was stoppered, the contents were mixed, and three 100 ml. portions were withdrawn for the dithizone determination. Then 13 liters of the urine was evaporated down in two large porcelain casseroles, the contents of one were finally transferred to the other, and the evaporation was completed. The stock urine was thoroughly mixed whenever a portion was withdrawn, and about midway in the evaporation two additional 100 ml. and one 200 ml. portions were withdrawn for additional dithizone determinations. After evaporation of the 13 liters, the material was charred upon the hot plate, and swelling was controlled by playing a small flame from a glass jet over its surface. When dry, the char was placed in a controlled muffle and ashed overnight at 500°C. The resultant gray



ash was worked into a paste with water plus 10 ml. of concentrated nitric acid. After drying down, the material was re-ashed for 30 minutes. The ash was then nearly white, but a little carbon persisted. The acid treatment was repeated with 5 ml. more of the nitric acid, and a white, carbon-free ash was obtained. It was treated with 300–400 ml. of water and 100 ml. of redistilled (constant boiling) hydrochloric acid, and the solution was boiled down slowly until bumping began. It was allowed to digest at steam bath temperature for several hours, and upon dilution the salts, with the exception of a slight siliceous precipitate, went into solution. This solution was filtered through a glass filter, and the precipitate was rinsed into a small platinum dish. Upon treatment with a few ml. of hydrofluoric acid the precipitate volatilized cleanly. The dish was rinsed with a small quantity of dilute hydrochloric acid, and the rinsings were added to the bulk of the filtrate, which was then made to 1 liter.

Values for lead in the stock urine were first established by the dithizone procedure on the 100 ml. portions and the one 200 ml. portion essentially as directed in a previous publication (14) and in *Methods of Analysis*, A.O.A.C., 1940. Because of slight manipulative changes suggested by subsequent experience the procedure applied here is given in detail. These changes alter in no way the general scope and principles of the method, and some of them have been indicated in the preceding discussion.

The samples were evaporated to dryness in porcelain and ashed overnight in some cases and from 2–3 hours in others. Neither ashing period yielded a clean ash so the casserole contents were treated with a little water plus 1–2 ml. of nitric acid. Upon drying down and reashing for 5–10 minutes a carbon-free ash resulted. It was taken up with water plus 10 ml. of constant-boiling hydrochloric acid, and the solution was evaporated nearly to dryness. An additional 10 ml. of hydrochloric acid was added, and the solution was re-evaporated nearly to dryness. Water plus a few drops of hydrochloric acid was then added, and the solution was heated upon the hot-plate to effect solution and transferred to a separating funnel with hot water. The volume at this stage was about 50 ml. One ml. of a 10 per cent hydroxylamine hydrochloride solution (deleaded by making a solution of 10 grams of the salt slightly ammoniacal, extracting out with dithizone in chloroform, washing with clear chloroform, acidifying slightly with redistilled hydrochloric acid, boiling to remove chloroform and making to 100 ml.) was mixed in, then 15 ml. of 50 per cent W/V citric acid solution, and the cooled mixture was made ammoniacal by the addition of redistilled ammonia. After the addition of 5 ml. of 10 per cent potassium cyanide solution the lead was extracted out with two successive portions of 15 ml. each of dithizone in chloroform of about 20 mg. per liter strength. (The dithizone was purified immediately before use (daily) by extracting out, once, 100 ml. of a chloroform solution of about this concentration

with 100–150 ml. of 1–100 ammonia, discarding the chloroform layer, acidifying the aqueous solution with hydrochloric acid, and re-extracting with 100 ml. of fresh chloroform). The dithizone extracts were “stripped” in a separatory funnel with 50 ml. of 1 per cent nitric acid containing in addition, 0.1 per cent of hydroxylamine hydrochloride (10), and the chloroform layer was drained off as cleanly as possible. The aqueous fraction was then washed with 10 ml. of pure carbon tetrachloride and the quantitative color development was made by adding the usual 10 ml. of ammonia-cyanide mixture and shaking out with 10 ml. of a purified carbon tetrachloride standard solution of dithizone of 12 mg./liter strength as noted above (0–10 mmg. range). The colorimetric solutions were read through a 2 inch cell on the neutral wedge photometer with filters 45, 51, and 56. After the reagent blank (about 1 mmg.) had been subtracted, the following values for lead (mmg. per liter) were returned on the 5–100 ml. samples and the one 200 ml. sample (last figure): 40, 39, 43, 35, 41, 42; average = 40 mmg./liter.

Auxiliary readings with filters 45 and 56 indicated no appreciable bismuth or tin interference. The result of 35 mmg. per liter is slightly low in comparison with the other values, but it was included in the average figure. This average result by the dithizone method was believed to be close to the true lead content of the stock urine, but in view of the criticism of Fairhall and Keenan it was desired to check this result by the Association's tentative electrolytic method. And because of danger of lead loss in the alkaline extraction of lead from the larger quantities of urine for reasons discussed later, it was believed that a sulfide method of isolation would more properly apply to the analysis of the stock urine ash solution. Accordingly, a 200/1000 aliquot (equal to 2.6 liters of urine) was placed in a glass-stoppered Erlenmeyer flask, 20 ml. of 50 per cent W/V citric acid solution was added, and the solution was brought to the incipient purple of bromophenol blue with ammonia. It was then treated with pure copper solution equivalent to about 5 mg. of copper and saturated with hydrogen sulfide. A clean sulfide precipitate was obtained and filtered off on a glass filter. It was dissolved in a few ml. of hot nitric acid, and the lead was isolated by a dithizone extraction and determined electrolytically. The full procedure is detailed in *Methods of Analysis, A.O.A.C.*, 1940 (7). As a check upon the electrolytic procedure the lead solution in the titrating vial was rinsed into a separatory funnel, and the lead was re-isolated by means of an additional dithizone extraction, stripped in a small quantity of 2 per cent nitric acid, and determined as chromate according to the procedure of Fairhall and Keenan (16). The electrolytic method returned a lead figure of 113 mmg., equal to 43 mmg. per liter on the original urine, and the corresponding value by the chromate method was 118 mmg., or 45 mmg. per liter. These values check quite closely with the value obtained by the colorimetric dithizone method. No reagent

blank was subtracted in obtaining the electrolytic value, and while one undoubtedly exists it was thought to be below the limit of detection of either the electrolytic or the chromate methods and was consequently ignored. In any event, the dithizone value, as contrasted with the values by the electrolytic or chromate procedures is not high, and readings with auxiliary filters 45 and 56 detected no interference. (Additional experiments were performed on the remainder of the stock urine ash solution as described later in this report.)

It was later decided to repeat this work and to make supplementary spectrographic and spectrophotometric tests on the colorimetric dithizone extracts and on the urine ash itself. The collection of a stock of urine was repeated (38 contributors) and analyzed as detailed above. Dithizone results on 25, 50, 100, 200, and 500 ml. portions were, respectively, 36, 30, 32, 30, and 35 mmg./liter. No appreciable interference was detected with filters 45 and 56. A further run on a 100 ml. portion withdrawn from the urine stock some days later yielded a value of 28 mmg./liter, but as some precipitation had ensued meanwhile this result is not included in the average of 33 mmg./liter.

The closely checking values noted above on sample volumes of from 25 to 500 ml. portions of this urine demonstrate the precision that can be attained with the dithizone method under carefully controlled conditions. These conditions presuppose no sample contamination, and meticulous evaluation and stability of the reagent blank, and they may not always obtain in routine analysis. The overall precision of the dithizone procedure on urine samples, with the 0-10 mmg. range and routine conditions, is perhaps  $\pm 0.5$  mmg. of lead. Thus with a 100 ml. sample, a urine of 30 mmg. per liter lead content may easily be reported as containing 25-35 mmg. per liter. Such figures are still satisfactory enough for toxicological interpretation, and precision would, of course, be doubled if 200 ml. samples were taken. In the present case, check values between 25 and 500 ml. sample volumes presuppose a precision of at least 0.2 mmg. However, the writer has obtained values of 68, 42, 35, 32, and 29 mmg. of lead per liter on samples of another urine stock of respectively 25, 50, 100, 250, and 500 ml. volume. These decreasing values indicate improper evaluation of the reagent blank (too low), and the trouble was believed to be due to the use of new "untempered" casseroles, which contributed only slight quantities of lead in blank runs but appreciably more when actual urine samples, whose salts are quite corrosive to porcelain, were ashed.

The dithizone figure for lead on the second batch of urine was checked by the sulfide-electrolytic and chromate methods as before, with results respectively, of 35 and 37 mmg. of lead per liter. These latter figures were on an aliquot of the urine ash solution equivalent to 3.75 liters of urine. The remainder of the ash solution was reserved for further experiments.

Again closely agreeing values were obtained by three methods differing entirely in principle. This in itself could be taken as proof that the dithizone values on the smaller quantities of urine are as reliable as any. The readings with the auxiliary 45 and 56 filters on the samples indicated normal or pure lead "colors," but it was considered that graphic and more complete proof of this could be afforded by a spectrophotometric comparison, throughout the visible range, of the final dithizone color, obtained from an actual sample of urine used in the lead evaluation, with that obtained from the indicated amount of pure lead as derived from the

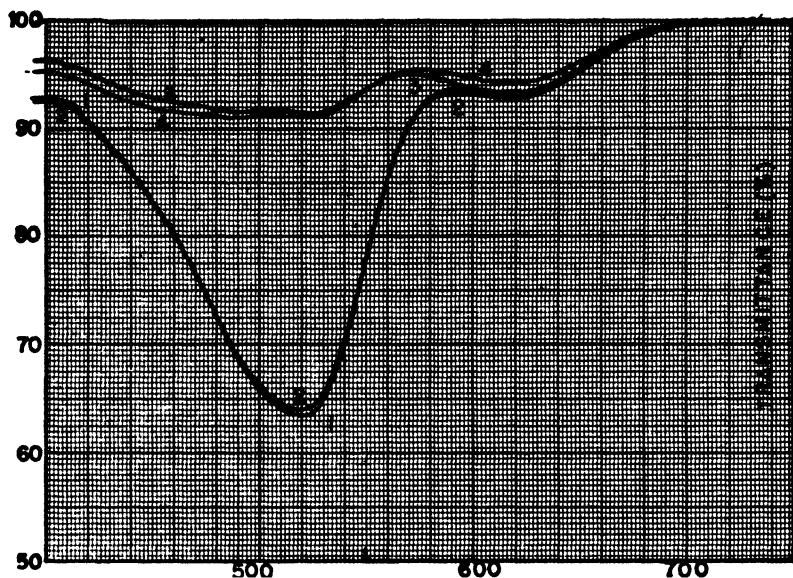


FIG. 5.—TRANSMISSION CURVES OF DITHIZONE "COLORS."

Curve 1. Obtained from sample of urine.

Curve 2. For same quantity of pure lead.

Curve 3. For "blank" of the determination.

Curve 4. For same quantity of lead as 3.

No. 51 filter reading. A General Electric recording spectrophotometer was available in the Cosmetic Division of the Food and Drug Administration and proved the ideal instrument for use in such studies. S. H. Newburger kindly cooperated by running these and other spectral curves mentioned in the course of this report.

Thus, a 200 ml. sample of urine was carried through the dithizone procedure as above, and the final colorimetric dithizone extract was read with filter 51. The indicated quantity of lead as pure lead nitrate was placed in a separatory funnel, and the dithizone color was developed as with a lead "standard"; both colors were then read through the same 1 cm. cell on the recording spectrophotometer (Figure 5). The curves are practically

coincident throughout the range. In the same figure the dithizone color from a blank run is compared in the same manner with the indicated quantity of pure lead. The differences noted at about 620  $m\mu$  with curves 3 and 4 and which are reversed at the blue end of the spectrum indicate a certain degree of dithizone decomposition in one case, with the appearance of a corresponding yellow phase. However, appreciable differences at these transmission levels reflect only negligible differences in concentration. It seems reasonable to assume that if some extraneous metal had been extracted along with lead from the actual urine sample, and especially if such interference, according to Fairhall and Keenan, may amount to several hundred per cent with urine, such close coincidence would not have been attained with curves 1 and 2. (Reference is made to differences in the spectral curves of the various dithizone complexes as described by Fischer and Weyl (20) and to their descriptions in Table 1.) Obviously the dithizone metal of curve 1 is lead.

For final confirmatory proof of the specificity of the dithizone-lead method, the Associate Referee made certain spectrographic tests on the second batch of stock urine to identify the naturally occurring metals and to compare these with the metals occurring in the final colorimetric dithizone extracts from which the photometric lead estimation is made. These extracts should contain no dithizone metals other than lead. In this spectrographic work the Associate Referee was fortunate in obtaining the advice and cooperation of B. C. Brunstetter and A. T. Myers of the Bureau of Plant Industry. The instrument employed was a large Bausch and Lomb Littrow spectrograph, and tests were conducted in the ranges of approximately 2500–3300 Å (position 5) and 3100–5900 Å (position 2), which ranges include sensitive lines of practically all the dithizone metals. Purest obtainable graphite electrodes were used in position 5 for the urine tests and pure copper electrodes for position 2.

To identify the metals naturally occurring in urine, 100 ml. of the second batch of stock urine was ashed in silica with the aid of 2.0 ml. of redistilled (from glass) nitric acid. The ash was then taken up with 5 ml. of 1+9 nitric acid. The figure for the lead content of this urine as determined above was used in an attempt to dry portions of this ash solution equivalent to 0.5 mmg. of lead in the electrode cups. However, the dried ash salts were so bulky that solution equivalent to only 0.33 mmg. of lead could be dried down in the cups of the graphite electrodes and to only 0.13 mmg. of lead in the copper electrodes.

To identify the metals in the final dithizone extracts, 100 ml. portions of the stock urine were ashed in porcelain and carried through the dithizone procedure as outlined above. The final colorimetric extracts were not read in the photometer, but they were run into Pyrex vials and the carbon tetrachloride solvent was evaporated. The residues were treated with a few drops of redistilled nitric acid, dried, and taken up as before

with 5 ml. of dilute nitric acid. This solution represented something over 3.0 mmg. of urine lead plus about 1 mmg. of blank lead for a total of about 4.0 mmg. Amounts of this solution calculated to contain approximately 0.5 mmg. of lead were evaporated down portion wise, in the electrode cups.

Another set of electrodes, prepared from the developed "colors" of 4.0 mmg. lead standards, was treated as above, and amounts of the resultant dilute nitric acid solution equal to 0.5 mmg. of lead were evaporated down in the electrode cups. A further set was prepared by evaporating down portions of a dilute nitric acid solution of pure lead nitrate equal to the same figure of 0.5 mmg. of lead. Finally, a set was prepared by carrying the apparatus and reagents of the procedure through blank runs, and on the basis of an expected total lead value of 1.0 mmg., evaporating down nitric acid solution of the colorimetric digest equal to 0.5 mmg. of lead. Graphite electrodes only were used with the last three sets of samples.

These spectrographic samples were arced with a sensitive plate (Eastman II-B), a current of 24 amperes at 120 volts D.C. being used with the graphite electrodes. The copper electrodes were mounted in water-cooled holders and arced at 8 amperes and 80 volts. The inter-electrode distance was 3 mm. and no external lens or sector was used. A mask 6 mm. high was placed in front of the collimating lens to reduce background caused by stray light. Along with the above samples salts of 15 of the more common dithizone metals were arced to simplify plate calibration and the identification of various spectral lines.

Noted in the spectrum of the urine ash itself, along with lead, were the lines of sodium, calcium, potassium, phosphorus, magnesium, aluminum, silicon, iron, manganese, copper, and traces of tin, vanadium, and bismuth. In the spectra of the dithizone extracts of the urine were found sodium, calcium, potassium, magnesium, silicon, aluminum, and lead along with traces of copper and possible traces of iron and bismuth. (The iron was probably an electrode impurity.) Three spectra of the dithizone extracts were followed on the plate (in order) by three of the extracted standards, three of pure lead nitrate, and three of the determination blank. As noted above all twelve strips were calculated to represent about 0.5 mmg. of lead, and all presented the same general appearance. Under visual inspection all the lead lines of the strips appeared of about equal intensity. A trace of silver was noted in one of the spectrograms prepared from the 4.0 mmg. "standards." Inspection of the spectra of the urine ash and the urine dithizone extracts run with the copper electrodes in position 2 revealed no additional metals in either. With the exception of zinc as noted below the most sensitive lines of the various metals were investigated.

Thus in summarizing the results of this spectrographic study, the Associate Referee considered that the traces of bismuth appearing in the final

dithizone extracts of this particular batch of urine were of most concern. A bismuth interference must certainly be guarded against in the dithizone analysis of mixed urine for lead; still, readings with auxiliary filters 45 and 56 had revealed no bismuth interference in the original analysis. Since the sensitivity of this method of bismuth detection has been fully investigated, as discussed above, it was thought that the traces of bismuth found in the spectrographic study of the dithizone extracts represented negligible amounts by comparison with the lead values. However, it was deemed advisable to recheck the efficacy of the differential filter method of interference detection, as regards bismuth, with actual runs on urine. Because the urine used in the spectrographic study now showed evidence of decomposition, a fresh stock was used in the following tests.

Three 100 ml. portions of the urine were carried through the dithizone procedure, No. 1 as collected, and with 0.5 and 1.0 mmg. of bismuth added, respectively, to Nos. 2 and 3. A normal curve for filter 45 was derived from the pure lead colors used in the dithizone standardization,

TABLE 6.—*Detection of bismuth interference in analysis of urine for lead*

NO.	FILTER 51	FILTER 45	"Pb"	NORMAL 45	"Pb"-BLANK (1-0 mmg.)	BI INDICATED?
			mmg.			
1	61.5	19.8	5.9	19.0	49 mmg./l	No
2	66.0	21.7	"6.4"	20.0	"54" mmg./l	Yes
3	69.0	23.6	"6.7"	20.5	"57" mmg./l	Yes

and normal readings were compared with those found on the three urine samples. Results are summarized in Table 6. It is noted that 0.5 mmg. quantities of bismuth per 100 ml. sample are detected, thus the spectrographic traces appearing in the urine extracts must have been less (probably much less) than represented by this amount.

The traces of copper noted in the extracts are probably due to use of this metal as a "gatherer" for lead in the sulfide purification of the citric acid employed in the dithizone procedure. Very small quantities must have remained in the citric acid to be carried mechanically into the dithizone extracts. Copper could not have been present as a dithizonate in the presence of cyanide. Use has been made of this ability to co-precipitate sulfides not only in this Association's methods for lead but also by Hubbard in his dithizone method for bismuth. In neither case has it been found to be a dithizone interference.

Zinc was not detected in the spectrographic analysis of the urine ash, but unless present in considerable amount it might not have registered in position 5, which does not include the most sensitive line, 2138 Å. However, the metal was certainly present, occurring either naturally in the urine itself or as contamination from the reagents and glassware used in

the preparation of the electrode sample. It was deemed advisable to make supplementary tests designed to prove its non-interference in the dithizone method, and because copper has also been mentioned as a possible dithizone interference it was decided to include it along with zinc in these tests.

Accordingly, two 200 ml. portions of urine were carried through the dithizone procedure for lead, and to one was added 0.1 mg. of pure copper and 0.1 mg. of zinc. The final dithizone colors of both samples were measured with the G.E. recording spectrophotometer on the same chart

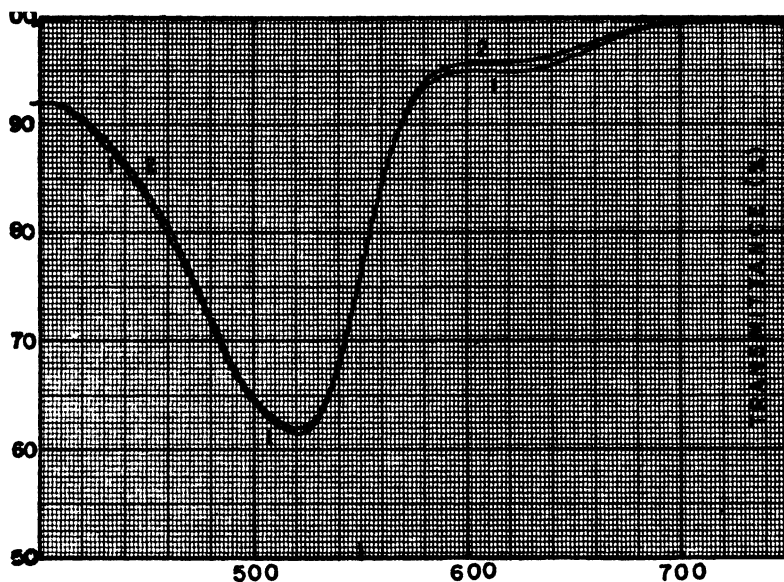


FIG. 6.—TRANSMISSION CURVES OF DITHIZONE "COLORS."

Curve 1. Obtained from sample of urine.

Curve 2. From same urine to which had been added Cu and Zn.

and through the same 1 cm. cell (Figure 6). The two curves are practically coincident throughout the spectrum, and this fact is definite proof that neither metal is an interference in the dithizone lead method.

The interference of zinc has been discussed (11). The Associate Referee has used the colorimetric dithizone method successfully on such products as maple sirup, where heavy contamination with zinc is the rule. The only instance in which zinc could be extracted in the presence of cyanide, in this experience, was when reactive sugar residues, liberated as a result of a strong acid treatment of unashed sugar products, combined with the cyanide (if present in insufficient amount) to allow a zinc-dithizone reaction (12). Even in this case the zinc coming through with the preliminary extracts was repressed by the cyanide present during the quantitative color development. Otherwise the presence of zinc may be ignored.



In further illustration of this point, in May, 1941, the Associate Referee used the colorimetric dithizone procedure in the analysis of zinc itself for lead and found no interference from the zinc. The sample was Bureau of Standards "Brass Special No. 110" and the certificate gave the lead content as 0.527 per cent (0.555 per cent cadmium). Approximately 0.5 gram was dissolved in nitric acid, and the solution was boiled and made to volume. When aliquots of this solution were given a preliminary dithizone extraction in the presence of sufficient cyanide and the lead was determined colorimetrically with a 0-100 mmg. range, the average of three closely agreeing determinations on aliquots of different size gave a lead figure of 0.532 per cent.

The remaining possibility suggested by Fairhall and Keenan in explanation of high results for lead with the dithizone procedure is that oxidation products of dithizone simulate the lead color to cause high lead values. The standard dithizone solution used in the final color development is probably meant here, for oxidation products in the dithizone solution used in the preliminary extraction would remain in the solvent phase when first extracts are shaken out with the stripping acid. The Associate Referee found that decomposition of the standard dithizone solution causes a reverse effect and that low lead values are obtained if this decomposition has become serious (10) and estimations are made, as usual, with a blue-green filter. Any slight deterioration of the standard dithizone solution is at once detected by reading the final extracts with an auxiliary blue filter, such as No. 45. To show the effect of dithizone decomposition upon the lead determination the following experiment was performed: A solution of dithizone in chloroform (the solvent used by most investigators) of about 4 mg. per liter strength was used in preparation of a 0-10 mmg. standard curve. The curve with filter No. 45 was also obtained. A portion of this standard solution was then oxidized to about one-half its original strength (one-half photometric density with filter No. 61) by shaking out with a very dilute aqueous solution of bromine water, and similar curves were prepared. It is noted from Figure 7 that the yellowish green oxidized solution yields lower values at all lead levels and especially so at the higher end of the range where the amount of dithizone was apparently insufficient to hold the lead in the chloroform phase and make the curve linear.

#### SOURCES OF ERROR IN THE DITHIZONE EXTRACTION

It thus appeared to the Associate Referee that all criticisms of the lead dithizone method had been investigated and its specificity, especially when applied to urine analysis, had been proven. Accordingly, it was thought that some other reason was accountable for the inability of Fairhall and Keenan to obtain check results with the dithizone and chromate methods. It was noted that while rejecting the dithizone reagent as a

means of quantitative estimation of lead, these writers employ it as a means of preliminary separation of lead prior to its determination as chro-

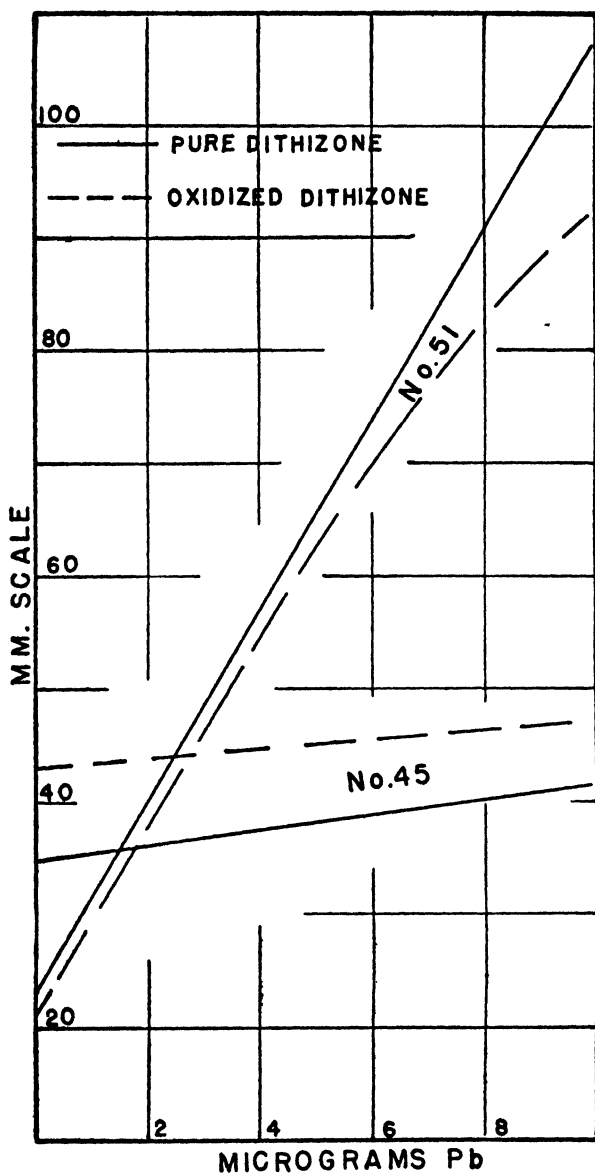


FIG. 7.—STANDARD CURVES FOR LEAD SHOWING EFFECT OF DITHIZONE OXIDATION.

mate. The dithizone extraction does afford a convenient means of lead isolation, but its use in this respect is definitely limited and must be

applied with caution (8). In the present case, unless the quantities of urine involved are limited, an ammoniacal solution of urine ash is prone to precipitate magnesium ammonium phosphate at a *pH* sufficiently high for efficient lead extraction in spite of the presence of large quantities of citrate. A mineral precipitate of any nature is apt to occlude lead and vitiate the dithizone extraction. In the experience of the Associate Referee the ash of one liter of urine is about all that can be safely handled if reasonable quantities, only, of citrate are employed to prevent formation of a precipitate. On the other hand, it has long been known that large quantities of citrate will repress the extraction of lead. Quantitative expression of this tendency has lately been published (2) (24). It appeared, therefore, that the analyst employing the dithizone method of isolation from the ash or digest of such large quantities of urine as were investigated by Fairhall and Keenan is caught between two possible sources of error; he must use a large excess of citric acid and in one case risk loss of lead through occlusion in an alkaline precipitate, or in the other, extract at such low *pH* that lead isolation is incomplete. Two series of experiments were made with the ash solutions prepared from the same two batches of urine to check the possibility of lead loss through these two sources of error.

In the first case the intent was to demonstrate lead loss through occlusion in an alkaline precipitate when the dithizone extraction is applied to the ash solution of the larger quantities of urine. Thus a 400/1000 aliquot of the solution from the first batch of urine (5.2 liters, lead content 43 mmg./liter) was placed in a separatory funnel, and 75 ml. of 50 per cent citric acid was added. The mixture was then made slightly ammoniacal, and because a precipitate began to form, 25 ml. more of the citric acid solution was added and the *pH* readjusted. Total citric acid present was 50 grams. Five dithizone extractions were then made with portions of 20 ml. each of dithizone in chloroform, the first two of 100 mg./liter and the others of 50 mg./liter strength. By the end of the extraction considerable precipitate had appeared in the funnel. The extracts were combined and washed thoroughly with dilute ammonia, and the lead was determined electrolytically.

As before, the lead figure was checked by a chromate precipitation and titration. The electrolytic procedure yielded 230 mmg. of lead and the chromate check 232 mmg., figuring respectively to 44 and 45 mmg. of lead per liter. The contents of the funnel were filtered through a glass filter, the funnel was rinsed, and the precipitate was dissolved in a small quantity of dilute hydrochloric acid. The resultant solution was tested for lead by adding citrate, sulfiding, and testing the solution from the sulfides for lead with dithizone. None was found, so it must be concluded that in spite of the appearance of a precipitate the lead was quantitatively removed by the dithizone extraction. A further experiment with a 200/1000 aliquot and 37.5 grams of citric acid likewise gave complete

recovery, but this quantity of citric acid did not prevent the appearance of an alkaline precipitate. However, when a 197/1000 aliquot was treated with only 15 grams of citric acid, made slightly ammoniacal, and immediately extracted as above, only 38 mmg. of lead appeared in the extract. The balance, 69 mmg., was recovered by filtering off the precipitate and determining occluded lead by the sulfide-extraction-electrolytic method. (These figures were checked by a chromate precipitation and titration as 40 and 66 mmg., respectively.) The total lead recovered by the electrolytic procedure was 107 mmg., or 42 mmg./liter of original urine.

Thus the possibility of serious lead loss through occlusion in an alkaline precipitate during the dithizone extraction of the larger quantities of urine ash is demonstrated. Fifteen grams of citric acid is inadequate for the ash solution of 2.5 liters of urine; 37.5 grams for this amount will not prevent the formation of a precipitate but will allow removal of lead provided the extraction is expedited. The quantity of citric acid used by Fairhall and Keenan is not stated in the paper under consideration, and it was at first thought that they had fallen into this error of alkaline occlusion of lead; however, it was established through personal interview that they had used 15 grams of citric acid per liter of urine and had at no time observed a precipitate during the alkaline extraction of their urine ashes or digests.

Thus it remained to check the feasibility of lead removal from urine ash solutions by the dithizone extraction with this proportion of citric acid at as high pH as possible without precipitate formation. Preliminary work with a urine salt base solution (9) proved that the operator could exceed the neutral point only slightly in the extraction, even with this large quantity of citric acid. Incomplete extraction was observed when attempt was made to isolate known quantities of lead from the purified (de-leaded) urine salt base at pH of 7.0 and slightly above. After the extraction seemed to be complete it was noted that if the alkalinity was increased by the addition of more ammonia and the solution re-extracted immediately, additional lead (as much as 60 per cent of the total added) was extracted in spite of the formation of an immediate precipitate.

Thus a 200/1000 aliquot of the second batch of urine ash solution (equivalent to 3 liters; lead content 35 mmg./liter) was treated with 45 grams of citric acid solution and brought to pH 7.2 (glass electrode) by the cautious addition of ammonia. The mixture was extracted three times with 50 ml. portions of dithizone in chloroform of 100 mg./liter strength. No cyanide was used, and the first extract was crimson, the second, bluish purple, and the third, green. The funnel was washed once by shaking out with a fourth portion of dithizone of 20 mg./liter strength. The solution was slightly hazy at the end of the extractions. The combined extracts were washed and electrolyzed, and only 39 mmg. of lead, not nearly enough to account for the color changes during the extraction, was re-

covered. To prove incomplete extraction, 50 ml. of the 100 mg./liter dithizone solution was added to the funnel, then 15 ml. of 10 per cent potassium cyanide and 10 ml. of strong ammonia, and the whole mixture was shaken out immediately. A copious precipitate formed during the shakeout but upon electrolysis the extract was found to contain 68 mmg. of lead. (Chromate checks upon these first and second extracts were, respectively, 38 and 66 mmg.) Further experiments showed that five extractions with the strong dithizone at pH 7.3 (about the critical point for precipitate formation) recovered only 86 per cent of the total lead. In the latter case there was no evidence of reaction during the last three extractions as the dithizone solution remained green. The presence of unextracted lead was confirmed as above by both the electrolytic and chromate procedures.

It is now believed that Fairhall and Keenan fell into the error of incomplete dithizone extraction of lead by applying this extractive separation at too low pH in the presence of excessive citrate. Incomplete extraction of lead may have been masked because of their non-use of cyanide, which would allow the extraction of zinc, which extracts at lower pH than does lead (29), with attendant color changes, thus giving the operator the false impression that the dithizone isolation was complete. The extractive separation of lead was probably inapplicable to the large quantities of material under test, and it is believed that had these authors employed a more suitable isolation procedure, such as the sulfide separation, their chromate results would have been higher and would have checked their dithizone values. Possibility of a bismuth interference in their dithizone results is discounted because of these authors' extensive experience with the urine analysis.

#### ADDITIONAL WORK

In view of the work of Biefeld and Patrick (2), H. J. Wichmann, the Referee on Metals in Foods, has suggested that tartrates may be as efficient in the prevention of an alkaline phosphate precipitate as are citrates, and at the same time may not suppress the dithizone extraction of lead. It was found, however, that tartrates were much less adequate for this purpose, and their use appeared to be infeasible. In the same way, no advantage was obtained from the addition of citrate as sodium citrate (thus, to avoid the effect of ammonium ion on a magnesium precipitate) and cautious adjustment of the ash solution to pH 8.0 with sodium hydroxide, instead of ammonia, prior to the dithizone extraction.

It appears, in résumé, that the lead-dithizone micro procedure can be applied with confidence except where a bismuth interference is anticipated. The photometric methods of detection of this interference discussed in this report are adequate, and where this interfering metal is found the

dithizone separation of Willoughby et al. (30) can be applied. Of special interest in connection with the bismuth separation is a recent paper by Bambach and Burkey (1), who routinely "strip" their preliminary extracts with an aqueous solution buffered at pH 3.4, instead of with the usual 1 per cent nitric acid. At this acidity their data show that the lead strips into the water phase, but that interfering bismuth remains in the dithizone layer and is so removed. Kluchesky et al. (25) employ a similar procedure. These ingenious modifications make the routine dithizone test very specific, and their incorporation into the Association's methods should be considered.

It may not be out of place in this report to note how the Laboratory of which Bambach and Burkey are associates has contributed to the advancement of micro methods for the determination of lead, and it is of further interest to point out that this Laboratory still retains the dithizone-lead procedure as a practical routine method after intensive investigation of the merits of other micro methods, notably the spectrographic, chromate, and polarographic procedures.

The Associate Referee believes that a fortunate choice was made in the selection of the dithizone method (1–200 mmg. of Pb), along with the electrolytic procedure (.05–10.0 mg. of Pb), as tentative lead methods. Since their proposal the subject of the micro-determination of lead in a great variety of products has been a most active one, yet both procedures have withstood their few criticisms remarkably well, and they will likely appear with only slight modification in the next edition of *Methods of Analysis*, A.O.A.C.

Remaining to be investigated is the cupferron separation of large quantities of iron and tin (11), sometimes encountered in the analysis of canned foods. Here large quantities of iron can interfere by oxidizing dithizone in the preliminary extraction, and tin can interfere at this stage by precipitating out and occluding lead. A simple method for their removal would be valuable. Exact methods for the determination of lead in oils and fats, which give trouble in both the ashing and digestion methods of sample preparation, also need further investigation. Acid aqueous extraction of the lead from solution of the oil or fat in some inert vehicle (kerosene, petroleum benzin) is probably the answer here, and preliminary work is encouraging. The procedure of Bambach and Burkey (1) should be further investigated to see if it applies equally well to larger quantities of lead and bismuth, and to note how the strength and volume of the dithizone solution used in the preliminary extraction may alter their clear-cut separation of lead and bismuth at pH 3.4. A similar separation of lead and thallium could be incorporated into the procedure if such a step is considered necessary.

Thus, work on the Association's lead methods appears nearly complete,

and the forthcoming revision of *Methods of Analysis*, A.O.A.C. should contain wholly satisfactory procedures for the micro-determination of this metal.

#### SUMMARY

The dithizone metals are enumerated, and certain properties of their complexes are listed. The metals interfering in the lead-dithizone determination are given, and photometric methods of detection of bismuth and tin interference are presented. The possibility of simultaneous determination of lead and bismuth is illustrated by various data, and a new procedure for the separation of small quantities of lead and thallium is outlined.

In view of certain criticisms of the lead-dithizone method, data on the analysis of urine are presented to confirm the fact that on small quantities the dithizone method yields results comparable with those given by other methods on much larger quantities. Spectrophotometric and spectrographic tests that show the specificity of the dithizone method are described, and specific tests prove the non-interference of copper, zinc, and oxidation products of dithizone.

Possible sources of error in the dithizone isolation of lead from large quantities of phosphatic material such as urine are illustrated.

Future work is outlined, and it is recommended that work on lead be continued.

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## REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural Experiment Station, College Station, Texas), *Referee*

Reports have been received from the following associate referees: A. L. Prince, on nitrogen; O. W. Ford, on potash and potassium recovery methods; H. R. Allen, acid- and base-forming quality; and W. Y. Gary, nitrogen and sulfur. Reports of W. H. Ross, on phosphoric acid, and of J. B. Smith, on manganese and magnesium, are not yet ready. No report will be made this year by Gordon Hart on calcium and sulfur.

High results on potash were obtained by a chemist who used porcelain dishes for ignition of the residue. Since the method does not state the kind of dish to be used, the text should be changed to limit these dishes to platinum or quartz.

According to N. D. Ellis, of the Kimble Glass Company, volumetric flasks used in the determination of total phosphoric acid in fertilizer may be etched by hydrochloric acid liberated in dissolving phosphates to such an extent as to affect the accuracy of the graduation. Tests were made by T. L. Ogier on 19 flasks that had been in use several years, with the results given in his paper (see p. 196). The capacity of these flasks was not affected so much as was expected, but the results show that the flasks should be tested after several years of use, and those found inaccurate should be graduated again or used when accuracy is not so important.

The methods for the determination of phosphoric acid in basic slag should be published with the other methods of phosphoric acid in *Methods of Analysis, A.O.A.C.*, 1940, instead of being separated by 14 pages. This can be done by making the following changes:

Delete 61. Add to 3 the words: "With basic slag, use 10 g of material." Delete 62 and 63. Add to 8: "With basic slag proceed as directed in 8(b)." Delete 64 and add to 9 the words: "With basic slag (then add remainder of 64)." Delete 65, and



add this paragraph to 12 as (c), preceded by the words, "In basic slag." Combine 66, 67, 68 into one paragraph and put immediately after 17 with the heading, "Citric acid-soluble phosphoric acid in basic slag—Official."

Final action is needed on a number of recommendations made last year, but this can not be done until the next meeting of the Association.

## REPORT ON NITROGEN\*

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

A collaborative study was made of the ferric sulfate dipotassium phosphate method for shortening the Kjeldahl digestion in the determination of total nitrogen in mixed fertilizers. This was recommended in last year's report (*This Journal*, 25, 322), following two years of preliminary work. The method, as tested by the Associate Referee, had been found to be rapid and accurate but possessed certain undesirable features when applied to routine work. Chief among these undesirable features was the large and cumbersome amount of salts produced in the digestion flask, and also the fact that the complete attention of the operator was required during the digestion period. It was felt, however, that the saving in time and gas might justify the use of this method, at least in specific instances.

Four mixed fertilizers varying from 2 to 8 per cent in total nitrogen were sent out to 10 collaborators, with the following instructions:

### INSTRUCTIONS TO COLLABORATORS

Analyze the four fertilizer samples for total nitrogen to include nitrates in triplicate by the following two procedures:

#### I. *Official Method:*

*Digestion Procedure.*—Place 1 gram of the sample in a Kjeldahl digestion flask. Add 30 ml. of  $H_2SO_4$  containing 2 grams of salicylic acid, allow to stand at least 30 minutes with frequent shaking, or until complete solution results, and then add gradually 2 grams of zinc dust (an unpalpable powder—granulated zinc or filings not satisfactory), shaking contents of flask at same time, and digest as follows:

Heat over low flame until all danger from frothing has passed. Increase heat until acid boils briskly and continue boiling until white fumes no longer escape from flask (5–10 minutes). Add ca. 0.65 gram of mercury and continue boiling until liquid in flask is colorless, or nearly so. If contents of flask are likely to become solid before this point is reached, add 10 ml. more of  $H_2SO_4$ . Complete distillation in the usual manner, using  $K_2S$  in the  $NaOH$  to precipitate the mercury. Also use a pinch of zinc dust in the distillation to prevent bumping.

#### II. *Rapid Method:*

*Digestion Procedure.*—Place 1 gram of the sample in a Kjeldahl digestion flask. Add 30 ml. of  $H_2SO_4$  containing 2 grams of salicylic acid. Allow to stand at least 30 minutes with frequent shaking, or until complete solution results, and then add 2 grams of zinc dust, shaking contents of the flask at the same time. Digest moder-

\* Journal Series paper of the New Jersey Agricultural Experimental Station, Rutgers University, department of soils.

ately for ca. 10 minutes. Then cool. Add ca. 0.65 gram of mercury, 10 grams of anhydrous dipotassium phosphate, and 6 grams of ferric sulfate. Turn the flame on full for a few minutes, and when the fumes appear to be all in the neck of the flask, turn the flame down slightly and control it so that the  $H_2SO_4$  does not distil. Always keep the flame higher than in the regular Kjeldahl digestion procedure. Rotate the flasks about every 5 minutes. The carbon will be oxidized in ca. 15 minutes, but continue the digestion for 30 minutes. (Due to the large amount of salts present, the contents of the flasks will be only semi-liquid, but no nitrogen is lost unless the material should go entirely dry.) Before the flasks are entirely cold, add the required quantity of water and shake. Some of the salts may still adhere to the sides of the flask but these will dissolve after the alkali is added. Use the regular amount of zinc dust in the distillation to prevent bumping, and  $K_2S$  in the  $NaOH$  to precipitate the mercury. After distillation, wash out the flasks before they get entirely cold.

The collaborators were as follows:

- (1) M. P. Etheredge, State College, Miss.
- (2) W. E. Dickinson, F. S. Royster Guano Co., Macon, Ga.
- (3) B. E. Plummer, Jr., Agr. Exp. Station, Orono, Me.
- (4) L. V. Crowley, Agr. Exp. Station, Amherst, Mass.
- (5) C. A. Butt and W. H. Banks, Internat. Minerals & Chem. Corp., East Point, Ga.
- (6) R. O. E. Davis, U.S.D.A., Bureau Plant Industry, Beltsville, Md.
- (7) H. C. Batton, Swift and Co., Fertilizer Works, Baltimore, Md.
- (8) R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
- (9) P. McG. Shuey, Shuey and Co., Savannah, Ga.
- (10) E. J. Deszyck, Agr. Exp. Station, Kingston, R. I.
- (11) A. L. Prince, *Associate Referee*

It should be noted that half of these collaborators are chemists in the laboratories of experiment stations and the other half in the laboratories of fertilizer industries.

TABLE 1.—Total nitrogen (%) (Av. of triplicate determinations from four fertilizer samples by the official and rapid methods)

COLLABORATOR	SAMPLE NO. 1		SAMPLE NO. 2		SAMPLE NO. 3		SAMPLE NO. 4	
	OFFICIAL	RAPID	OFFICIAL	RAPID	OFFICIAL	RAPID	OFFICIAL	RAPID
1	2.17	2.15	4.40	4.47	8.34	8.37	5.82	5.80
2	2.26	2.27	4.50	4.47	8.47	8.47	5.93	5.89
3	2.19	2.22	4.40	4.33	8.34	8.21	6.03	6.07
4	2.15	2.17	4.62	4.52	8.19	8.16	5.94	5.92
5	2.27	2.30	4.43	4.43	8.50	8.48	5.86	5.89
6	2.19	2.16	4.16	4.13	8.33	8.20	5.80	5.82
7	2.24	2.19	4.34	4.44	8.42	8.27	5.91	5.90
8	2.07	2.10	4.31	4.25	8.10	8.07	5.71	5.75
9	2.24	2.23	4.39	4.39	8.23	8.23	5.97	5.95
10	2.09	2.16	4.47	4.45	8.23	8.17	5.93	5.94
11	2.19	2.11	4.15	4.23	8.34	8.27	5.91	5.94
Average	2.19	2.19	4.38	4.37	8.32	8.26	5.89	5.90

The collaborative results comparing the official and rapid method for total nitrogen on four of the mixed fertilizer samples are reported in Table 1.

Comparing the collaborative data on the rapid and official methods, the Associate Referee found the greatest individual deviation from the official method to be  $\pm .07$  per cent for Sample 1,  $\pm .08$  per cent for Sample 2,  $\pm .13$  per cent for Sample 3, and  $\pm .04$  per cent for Sample 4. When the 11 collaborative results were averaged, the differences between the rapid and official methods were zero for Sample 1,  $-.01$  per cent for Sample 2,  $-.06$  per cent for Sample 3, and  $+.01$  per cent for Sample 4. Furthermore, the deviation from the mean for any one sample was no greater by the rapid method than by the official method. These results establish the accuracy of the rapid digestion method on mixed fertilizers as compared to the official method. However, the manipulative features of the rapid method are very important to consider if this method is to be used in control laboratories. The comments by the collaborators in regard to this method should be seriously considered.

#### COMMENTS BY COLLABORATORS

1.—Experience such as we were enabled to gain from the examination of your samples leads us to believe that the "Thio" method is to be preferred. We believe that it gives better checks and involves less danger of "going dry."

2.—The determination seems to require too much individual attention for acceptance in a routine laboratory. Does not seem to be the sort of determination one may start off and then leave for an hour or forget for another hour without disaster. The acid does not seem to condense in the flask and wash down adhering organic matter, as in the case of regular Kjeldahl. I am wondering if the method would not be a good substitute for the old permanganate addition at the end of the Kjeldahl digestion? Run the regular Kjeldahl for 45 minutes, and then add the phosphate and  $\text{Fe}_2(\text{SO}_4)_3$  for the last 15 minutes of digestion.

3.—The "rapid method" proved to be satisfactory to operate, and the average results obtained checked reasonably well with those obtained by the official method. The time of digestion is materially shortened by the "rapid method" and would be of importance when results of analyses were needed in a hurry. In our regular analysis for nitrogen on fertilizer samples in our control work, the chemist's work is so arranged, having about three sets in different stages of operation at one time, that he is busy all the time, and the time saved in digestion probably would not increase his total number of determinations.

4.—The rapid method requires more attention during digestion. It is questionable whether actual working time is reduced, especially if electricity is the sole source of heat, for nitrogen digestions and distillations as it is in this laboratory. Once the units have been heated it is impossible to cool them quickly merely by turning off the source of heat. Under these conditions even the total time required for completion of an analysis is not shortened materially. Blanks found by using  $\text{Fe}_2(\text{SO}_4)_3$  and dipotassium phosphate were appreciably higher.

5.—Our results indicate that the two methods give practically the same results. While the official method is longer, it requires less attention and for this reason we prefer this method.

7.—It was found that the rapid method of digestion carried an abnormally high blank.

8.—Prefer the use of 10 grams of  $K_2SO_4$  containing .7 gram of  $H_2O$  to either of the methods described above.

9.—The quick method appears to give very good results on all samples, but it seems to us that no great amount of time is saved when it is employed on complete fertilizers or materials like cottonseed meal or fish scrap, which are easily digested anyway. I consider that the rapid method is quite advantageous on materials that require long digestion, such as process tankage. Personally, I should prefer to see the rapid method adopted as an auxiliary method rather than to replace the regular A.O.A.C. method.

10.—I find the new procedure very satisfactory, and for a hurried determination it is a great time saver. However, because of our variable heating facilities, I could not always attain complete oxidation in the time allowed by the method.

It is quite evident from the collaborators' comments that the rapid method is not very popular, at least for control work, and the Associate Referee also considers that most of the criticisms made were justifiable. The original intention, however, was not to replace the official method with the rapid method but to offer it as an alternative for special cases if it seemed feasible. Since the method does not adapt itself very well to control work, further work on this method might be discontinued.

During the course of the year another rapid digestion procedure, involving the use of perchloric acid, was tried out with considerable success. The method is to appear soon in the *J. Ind. Eng. Chem., Anal. Ed.*, under the title, "A Rapid Kjeldahl Digestion Method Using Perchloric Acid," by L. P. Pepkowitz,\* A. L. Prince, and F. E. Bear. In this method a preliminary 10-minute digestion is carried out, selenium oxychloride being used as the catalyst. The oxidation is finally completed by heating the solution under definite conditions with 0.5 ml. of 35 per cent aqueous solution of perchloric acid. The average digestion time for 1 gram samples is approximately 30 minutes. Organic fertilizers, as well as mixed fertilizers, composts, and soils gave results by this method that were in close agreement with the official method. Besides being rapid, this digestion procedure is free from the obnoxious feature of the use of large quantities of salts. The main difficulty of the method from the control chemist's point of view would be the amount of attention required for the careful heating of the perchloric acid mixture.

In order to obtain further preliminary information concerning this method, L. P. Pepkowitz and the Associate Referee determined the total nitrogen in the four collaborative samples of mixed fertilizers by the perchloric acid method. The results, compared with the average results of the collaborators by the other two methods, are recorded in Table 2. The data for the perchloric acid method seem to compare quite favorably with either the official or rapid salt method.

In Table 3 is shown a comparison of all three methods on a sample of

\* Research Assistant in Plant Physiology, New Jersey Agr. Exp. Station.

TABLE 2.—Total nitrogen in 4 mixed fertilizer samples by three methods (per cent)

	SAMPLE 1			SAMPLE 2			SAMPLE 3			SAMPLE 4		
	OFFICIAL	RAPID	PER-CELOREX	OFFICIAL	RAPID	PER-CELOREX	OFFICIAL	RAPID	PER-CELOREX	OFFICIAL	RAPID	PER-CELOREX
Analysis received from Virginia-Carolina Chem. Corp.	2.24			4.17			8.48			6.00		
Average of 11 collaborators	2.19	2.19		4.38	4.37		8.32	8.26		5.89	5.90	5.89
A. L. Prince	2.19	2.11	2.24	4.15	4.23	4.12	8.34	8.27	8.16	5.91	5.94	6.00
L. P. Pepkowitz			2.13			4.19			8.19			6.00

TABLE 3.—*Total nitrogen in process tankage by three methods (per cent)*

ANALYST	OFFICIAL METHOD	RAPID METHOD	PERCHLORIC ACID
P. McG. Shuey	9.00	9.08	
L. P. Pepkowitz			8.87
A. L. Prince	8.90	8.98	8.91

process tankage, which is usually considered to be difficult to digest, requiring 2½–3 hours by the official method.

Further preliminary work with the perchloric acid method, especially as applied to control work, is contemplated by the Associate Referee.

## REPORT ON POTASH\*

By O. W. FORD (Purdue University Agricultural Experiment Station,  
West Lafayette, Ind.), *Associate Referee*

In accordance with the recommendations of the Association (*This Journal*, 25, 47–50), referee work was conducted this year by collaboration. A copy of the proposed work was sent to each of 28 chemists who had expressed a willingness to collaborate.

### COLLABORATIVE WORK ON POTASH IN FERTILIZERS

It is recommended—

(6)† “That the study of methods for the recovery of platinum be continued.”

*Suggested procedure.*—Read the condensed and revised platinum recovery method enclosed and express your opinion as to the advisability of this or some modification of it being recommended as a tentative method to the A.O.A.C. It is desired that all collaborators report on this part.

(31) “That the method of washing by decantation given for the determination of potash (42, p. 31)‡ be further studied.”

*Suggested procedure.*—Make sufficient solution of Sample A (0-0-45) according to 41(a), p. 31, to permit the making of 12 potash determinations. In 42(a), p. 31, proceed as usual to “evaporate on a water bath, etc.” At this point add to the first 6 determinations the usual 6 ml. of 80% acid-alcohol (acid and alcohol should have been previously mixed and cooled to 20°C.), allow to stand 15 minutes, transfer directly to the glass sinter or asbestos padded Gooch, using a policeman to break up the salt crystals and aid in the transfer in case the  $K_2PtCl_6$  salt sticks to the dish, and continue the washing on the filter until 75 ml. of the 20°C. cooled alcohol has been used (making sure that the filtrate is colorless). Wash with 5 (10 ml.) portions of  $NH_4Cl$  to remove the impurities and then with 5 (10 ml.) portions of the cooled alcohol; dry at 100°C., weigh, and calculate to  $K_2O$  (precipitate should be completely soluble in water).

Treat the second 6 determinations as directed above except to wash the salt in

\* Journal Paper No. 55 of the Purdue University Agricultural Experiment Station.

† Paragraph numbers refer to recommendations of committee, *This Journal*, 25, 47 (1942).

‡ *Methods of Analysis*, A.O.A.C., 1940.

the dish by decantation with 5 (10 ml.) portions of the 20°C. cooled alcohol, using a policeman to break up the crystals and to loosen from the dish. Then transfer the potash salt to the sinter or Gooch, using not more than 25 ml. of the cooled alcohol. Follow with a wash of 5 (10 ml.) portions of  $\text{NH}_4\text{Cl}$  and finally with 5 (10 ml.) portions of the cooled alcohol; dry and weigh as directed above, making correction for insoluble residue if necessary. Approximate  $\text{K}_2\text{O}$  value of Sample A, 45.0. Number of potash determinations, 12.

(41) "That the collaborative work to determine the effect of temperature on the solubility of  $\text{K}_2\text{PtCl}_6$  in acid-alcohol in the determination of potash by the official method be continued."

*Suggested procedure.*—Prepare sufficient solution of Samples A and B according to 41(a), p. 31, to make 12 potash determinations of each sample by 42(a), p. 31, with the following modifications: Determine the potash in the first 6 samples by cooling to about 20°C. both the acid-alcohol and the 80% alcohol used as wash in the determinations. Determine the potash in the second 6 samples by warming to 30°C. both the acid-alcohol and the 80% alcohol. In each case, use 75 ml. of acid-alcohol and alcohol for the wash preceding the  $\text{NH}_4\text{Cl}$  wash and 50 ml. of 80% alcohol for that following it, and break up the potash salt with a policeman. Keep the acid-alcohol mixture while in contact with the potash salt for the 15 minute period at the proper specified temperature (either 20° or 30°C.).

Approximate $\text{K}_2\text{O}$ value of Samples A	45.0
B	20.0

Number of potash determinations 24

(42) "That the official method for the determination of potash be studied to determine the effect of adding  $\text{H}_2\text{SO}_4$  at the beginning of the evaporation instead of near the end, 42(a), p. 31."

*Suggested procedure.*—Prepare sufficient solution of Samples B and C to make 12 potash determinations of each. Determine the potash in the first 6 samples as directed in 42(a), p. 31, evaporating the aliquot to dryness before adding the  $\text{H}_2\text{SO}_4$  and burning off. Determine the potash in the second 6 samples as directed in 42(a), p. 31, but adding the  $\text{H}_2\text{SO}_4$  before the aliquot has been evaporated to dryness.

Approximate $\text{K}_2\text{O}$ value of Sample B	20.0
C	9.0

Number of  $\text{K}_2\text{O}$  determinations 24

Those reporting collaborative potash results for par. 31, 41, and 42 should follow the form outlined below:

1. Type of filter . . . . . (a) If glass sinter list porosity number, (b) asbestos padded Gooch, (c) special padded Gooch, or (d) . . . . .?
2. Volume of alcohol used for washing (ml.).
3. Temperature of alcohol used for washing (°C.).
4. Type of dish used for ignition: Pyrex, porcelain, platinum, or . . . . .
5. Capacity of dish used for ignition (ml.).
6. Size of aliquot used for determination ( $\frac{1}{4}$  or  $\frac{1}{2}$  gram).
7. Evidence of visible insoluble residue.
8. State if results were obtained by direct weighing, . . . . . or by dissolving out the  $\text{K}_2\text{PtCl}_6$  and weighing back . . . . .
9. List the individual results as well as the average.

#### COMMENTS ON RECOVERY OF PLATINUM

In 1942, as has been the case for the past three years, very few of the collaborators took time to try out the platinum recovery and purification methods sent out to them. Several of them commented favorably in that

they thought that the A.O.A.C. should provide a method for the recovery and purification of platinum but that at present they were so well pleased with their own procedure that they felt they should not take the time to try out the condensed method submitted. One commercial chemist stated that platinum recovery and purification was a serious problem in his laboratory and that he thought that it was a good thing to study this problem and submit something to the Association. A few stated that in

TABLE 1.—*Study of method of washing  $K_2PtCl_6$  in determination of  $K_2O$  in fertilizers*  
Sample transferred and washed on filter (Method A) versus Sample washed by decantation from dish (Method B)  
Sample A (0-0-45)

ANALYST NO.	ANALYSES MADE	METHOD A			METHOD B		
		HIGH	LOW	AV.	HIGH	LOW	AV.
2	6	42.80	42.64	42.73	42.84	42.66	42.74
7	6	42.92	42.76	42.83	42.84	42.74	42.77
8	6	43.10	42.78	42.91	43.20	42.87	43.08
9	6	42.92	42.80	42.88	42.96	42.76	42.85
10	6	43.32	43.12	43.20	43.24	43.12	43.16
12	6	43.06	42.75	42.94	43.10	42.83	42.99
13	6	43.17	42.94	43.05	43.17	42.99	43.06
17	3	42.80	42.75	42.77	42.85	42.75	42.78
20	6	43.19	43.00	43.09	43.09	42.88	42.94
21	6	43.28	43.19	43.23	43.17	43.09	43.12
25	6	43.07	42.49	42.83	42.85	42.51	42.69
14*	6	43.12	43.01	43.07	43.09	42.98	43.03
Average		43.06	42.84	42.95	43.03	42.84	42.93
Max. Variation		.52	.70	.50	.40	.61	.47

\* Entered after chart was typed, not included in averages.

part the condensed method was similar to that being used in their laboratory at the present time.

In view of the lack of collaborative response for the past three years, even though some interest has been indicated, the Associate Referee considers that something definite should be presented to the Association and with this thought in mind, the condensed method sent out in 1942 will be recommended to the Association as a tentative method for the recovery and purification of platinum used in the determination of potash in commercial fertilizers.

#### COMMENTS ON DATA IN TABLES 1 AND 2

The work of 11 of 28 chemists who signified a desire to collaborate on the potash work is reported in Table 1. Although good agreement was



TABLE 2.—*Effect of temperature of acid-alcohol and alcohol on determination of K<sub>2</sub>O in fertilizers*

Washed with alcohol at 20°C. (Method A) versus Washed with alcohol at 30°C. (Method B)  
Sample A (0-0-45)

ANALYST NO.	ANALYSES MADE	METHOD A			METHOD B		
		HIGH	LOW	AV.	HIGH	LOW	AV.
2	6	42.90	42.82	42.85	42.92	42.82	42.87
7	6	42.86	42.72	42.79	42.82	42.76	42.79
8	6	43.30	42.82	43.08	43.44	43.06	43.19
9	6	42.84	42.76	42.79	42.76	42.60	42.65
10	6	43.72	43.44	43.64	43.32	43.20	43.24
12	6	43.02	42.91	42.94	43.14	42.87	42.98
13	6	43.26	43.05	43.14	43.21	42.83	43.04
17	3	41.95*	41.75*	41.85*	41.70*	41.55*	41.62*
20	6	43.02	42.80	42.88	43.21	42.97	43.11
21	6	43.16	43.06	43.11	43.08	43.00	43.06
25	6	43.07	42.49	42.83	43.10	42.68	42.53
14†	6	43.12	43.01	43.07	43.14	43.07	42.98
Average		43.12	42.89	43.01	43.10	42.88	42.95
Max. Variation		0.88	0.95	0.85	0.68	0.60	0.66
Sample B (0-20-20)							
2	6	19.52	19.40	19.46	19.67	19.61	19.64
7	6	20.36	20.20	20.29	20.30	20.20	20.25
8	6	20.18	20.06	20.12	20.12	20.01	20.05
9	6	19.84	19.68	19.75	19.72	19.56	19.64
10	6	20.68	20.66	20.68	20.76	20.64	20.69
12	6	20.15	20.00	20.07	20.12	20.00	20.03
13	6	20.65	20.46	20.55	20.66	20.57	20.61
17	3	18.70*	18.60*	18.67*	18.60*	18.50*	18.55*
18	1	—	—	19.54	—	—	19.54
20	6	19.95	19.88	19.93	19.94	19.85	19.90
21	6	20.04	19.88	19.96	19.96	19.82	19.89
14†	6	20.19	20.08	20.02	20.04	20.03	20.01
Average		20.15	20.02	20.04	20.14	20.03	20.02
Max. Variation		1.16	1.26	1.22	1.09	1.03	1.15

\* Omitted from averages and maximum variation.

† Entered after chart was typed, not included in averages.

obtained by all the chemists using both of the methods of washing the potassium chloroplatinate precipitate, no preference was expressed for either procedure. The Associate Referee in last year's report referred to the fact that not all the chemists were using either the same concentration of alcohol or the same quantity for the washing of the precipitate. Therefore a definite concentration of alcohol (80% by volume), a definite tem-

perature level (20°C. versus 30°C.), and a definite volume of alcohol per determination (125 ml.) were indicated in this year's method of procedure sent to the various chemists. It is considered that the good agreement obtained by most of the chemists is largely due to the work being done under more nearly the same laboratory conditions than has been the case of reports filed in previous years. Part of this same thought was expressed by Magruder\* in 1931 and 1932. A resume of Magruder's reports for December of 1931 and January and February of 1932 would indicate a definite need for more explicit directions regarding the concentration and the amount of alcohol to be used per determination if concordant potash results are to be obtained. In Magruder's December, 1931, statement, the volumes of alcohol reported by various chemists ranged from 10 to 225 ml., and the results for this month were not so concordant as they were for the January and February reports of 1932, when more definite volumes of alcohol were requested. Higher potash values were reported when 95 per cent alcohol replaced 80 per cent. Ford and Hughes<sup>1</sup> have reported additional work along this line but under very definite conditions. Mitchell and Ford<sup>2</sup> investigated the purity of the potassium chloroplatinate obtained with the use of alcohols of 80 per cent and stronger (80, 85, 90, and 95 per cent). They found that within the limits of experimental error the increased potash value obtained by using stronger alcohol was all potassium chloroplatinate. For this reason there should be no objection to the use of stronger than 80 per cent alcohol and acid-alcohol for the determination of potash in fertilizers.

The Associate Referee considers that this matter should be cleared up by a definite recommendation relative to concentration of alcohol and the amount of alcohol to be used per determination for potash work.

In Table 2 appear the results of 11 of the 28 collaborators that were asked to report on this part of the work. Here, again, the agreement, while not so good as that reported in Table 1, is much better than that received in any of the past few years. However, it is considered that the agreement is for the most part due to the more explicit directions submitted. When the directions were sent out it was recognized that it would be hard to obtain results from the various chemists that would substantiate work reported by Hughes and Ford<sup>3</sup>, as they reported on solubilities at temperatures much higher than those indicated in the procedure. From reports that have come to the Associate Referee, the average temperature for most of the laboratories doing potash would probably fall between 20° and 30°C. most of the year. This being the case, the work should be done as nearly under these conditions as possible. Even then, with careful

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\* Results of check fertilizer analyses on samples submitted by E. W. Magruder.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 217 (1942).

<sup>2</sup> *In press.*

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 233 (1941).

TABLE 3.—*Effect of time of addition of  $H_2SO_4$  on determination of  $K_2O$  in fertilizers  $H_2SO_4$  added at start of evaporation (Method A) versus  $H_2SO_4$  added near end of evaporation (Method B)*

ANALYST NO.	ANALYSES MADE	METHOD A			METHOD B		
		HIGH	LOW	AV.	HIGH	LOW	AV.
Sample B (0-20-20)							
2	6	19.61	19.52	19.57	19.60	19.57	19.60
7	6	20.34	20.24	20.29	20.32	20.24	20.28
8	6	20.04	19.83	19.96	20.16	19.98	20.04
9	6	20.00	19.88	19.95	19.84	19.68	19.75
10	6	20.70	20.65	20.68	20.71	20.66	20.69
12	6	20.13	20.01	20.08	20.05	19.97	19.99
13	6	20.67	20.43	20.59	20.67	20.49	20.58
17	3	19.85	19.80	19.82	19.85	19.80	19.82
20	6	20.01	19.80	19.86	19.90	19.80	19.84
21	6	20.04	19.94	19.97	20.04	19.96	19.98
14*	6	20.19	20.08	20.00	20.14	20.07	19.97
Average		20.14	20.01	20.08	20.11	20.02	20.06
Max. Variation		1.09	1.13	1.11	1.11	1.09	1.09
Sample C (3-18-19)							
2	6	9.48	9.40	9.43	9.46	9.38	9.44
7	6	9.78	9.53	9.65	9.61	9.50	9.55
8	6	8.78	8.62	8.72	9.03	8.72	8.89
9	6	9.42	9.26	9.31	9.46	9.30	9.37
10	6	10.10	9.92	10.04	10.11	9.99	10.06
12	6	9.22	9.11	9.16	9.22	9.01	9.15
13	6	9.60	9.43	9.51	9.65	9.51	9.58
17	3	9.40	9.30	9.37	9.50	9.40	9.43
20	6	9.39	9.31	9.34	9.42	9.27	9.34
21	6	9.56	9.42	9.49	9.56	9.44	9.48
14*	6	9.63	9.61	9.57	9.49	9.46	9.42
Average		9.47	9.33	9.40	9.50	9.35	9.43
Max. Variation		1.32	1.30	1.32	1.08	1.27	1.17

\* Entered after chart was typed, not included in averages.

work, the analyst should be able to determine the difference in solubility of the potassium chloroplatinate in 125 ml. of alcohol at 20° and 30°C., and when most of the reports were analyzed carefully it was found that such was the case. A few, however, showed a greater variation in potash results made on the same solution at one temperature than in those made at varying temperatures. This may be due to faulty or poor equipment, which the Associate Referee hopes is the case, as this factor can usually be readily remedied.

The differences between the average potash values at 30°C. and 20°C.

were not great enough to be significant. However, the fact that the values at 30°C. were slightly lower than those at 20°C. is significant. Previous work had shown the difficulty of demonstrating any difference at all under existing laboratory conditions. Therefore it is recommended that a statement relative to temperature be inserted in the method.

#### COMMENTS ON TABLE 3

In Table 3 appear the results of ten chemists on the two samples sent out for this study. Some prefer adding the sulfuric acid at the start, while others prefer adding it at the end of the evaporation. A study of the results indicates, at least on the two samples tried, that it would not make any difference. Although in nearly every case there was good agreement between the individual results, there was more variation between the individual results by one method than there was between those by both methods.

#### LIST OF COLLABORATORS

- (2) Katharine W. Ford and C. C. Howes, Davison Chem. Corp., Baltimore, Md.
- (7) R. O. Powell and F. B. Carpenter, V. C. Chem. Corp., Richmond, Va.
- (8) Marvin H. Snyder, Dept. of Agr., Charlestown, W. Va.
- (9) H. Robert DeRose and Philip H. Smith, Mass. State College Agr. Exp. Station, Amherst, Mass.
- (10) Wm. Chapman, Arthur Roberts, and R. L. Johnson, Consolidated Rendering Co., Boston, Mass.
- (12) R. C. Koch, Swift & Co., Fertilizer Works, Hammond, Ind.
- (13) H. C. Batton, Swift & Co., Fertilizer Works, Baltimore, Md.
- (14) M. P. Etheredge and W. F. Hand, Mississippi State College, College Station, Miss.
- (17) A. N. Lineweaver and E. W. Magruder, F. S. Royster Guano Co., Norfolk, Va.
- (18) P. M. Shuey, Shuey & Co., Savannah, Ga.
- (20) H. E. Witt and W. R. Austin, Armour Fertilizer Works, Nashville, Tenn.
- (21) W. E. Dickinson, F. S. Royster Guano Co., Macon, Ga.
- (25) R. L. Willis, Agr. Exp. Sta., New Brunswick, N. J.

#### COMMENTS OF COLLABORATORS

2.—Results were negative in all three of the experiments. Being satisfied with present platinum recovery method, I did not try out the proposed methods. Used 100–150 ml. of 95% alcohol. Alcohol used at room temperature, platinum dishes of 100 ml. capacity,  $\frac{1}{4}$  gram aliquots, no visible residue, results by direct weighing.

7.—Have tried platinum methods but still like our own best.

8.—It might be of interest to determine whether there is a wider variation in the results within the different methods or between the different methods.

9.—Used glass sinter, porosity (M), 130 ml. alcohol per determination, and platinum dish of 100 ml. capacity,  $K_2PtCl_6$  dissolved out and weighed back.

10.—Dilute nitric acid seemed to dissolve some platinum. Have no preference in platinum recovery method except dislike loss of platinum in dilute nitric acid. Method of preparation is our regular method. Used asbestos padded Gooch and 125 ml. of alcohol for washing (platinum dish, 100 ml. capacity). There was no visible residue, and results were obtained by direct weighing. Temperature differ-

ences on low potash goods are not noticeable, but they are on high potash goods.

12.—Glass sinter of medium porosity and 135–140 ml. of alcohol used in washing silica dishes of 100 ml. capacity. Visible residue detected in all determinations; results by dissolving the  $K_2PtCl_6$  and reweighing, platinum reduced in acid medium. Method of purification all right as stated in directions.

13.—Glass sinter of medium porosity and 150 ml. alcohol used for A + B and 125 ml. for C sample. Temperatures as indicated; platinum dishes, capacity 75 ml. Visible insoluble residue in all samples; results by dissolving out and weighing back.

14.—Used asbestos padded Gooch and 125 ml. of alcohol, 20° and 30°C., as directed. Also used platinum dish, 100 ml.,  $\frac{1}{2}$  gram aliquot, no visible residue, and direct weighing.

17.—Used porcelain Gooch, paper, and asbestos pad, 125 ml. alcohol, temperature as directed, platinum dish of 75 ml. capacity, and a 0.5 gram sample. There was no visible residue. Results were obtained by direct weighing. No differences were recognizable in Sections 31 and 42. Lower results obtained at the higher temperature.

18.—One determination of each part with Sample B, Section 41, gave same value at 20° and at 30°C. There was no time for further work.

20.—Have own method of platinum recovery, and consider platinum recovery very important. Results were obtained by dissolving out and reweighing. Used asbestos-padded Gooch. Temperature above 35°C. may dissolve some  $K_2PtCl_6$ . Platinum dishes, 80 ml., were used. In the laboratory both platinum and silica dishes are normally used. We use fractional pipets in our work. No visible residue was detected. We favor the use of at least 85% alcohol as 80% isn't strong enough.

21.—Asbestos-padded Gooch, 150 ml. of alcohol, temperatures as indicated, 50 ml. platinum dish and  $\frac{1}{2}$  gram aliquot were used. There was no visible residue. Results were obtained by direct weighing.

25.—Asbestos-padded Gooch,  $\frac{1}{2}$  gram sample, 125 ml. alcohol and porcelain dish of 110 ml. were used. Results were obtained by dissolving out and reweighing.

#### ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to D. M. Doty and H. L. Mitchell of the Agricultural Chemistry Department, Purdue University, for valuable suggestions and criticisms in the development of the investigations covered by this report.

#### REPORT ON COPPER AND ZINC IN FERTILIZERS

By W. Y. GARY (Department of Agriculture, Tallahassee, Fla.),  
*Associate Referee*

The methods that were presented at the 1941 meeting of the Association were used in this laboratory during the past year but no collaborative work was done. Satisfactory results, in most cases, continued to be obtained by the methods as written, but in a few cases it was found that slight changes made some improvements.

No change was found advisable in the long volumetric method for the determination of copper (*This Journal*, 24, 67), adopted as official (first action) last year. This method was tried two years by collaborators, and

no revision with conclusive proof was suggested. The method is accurate and not subject to interference by any element found in fertilizers.

The short volumetric method for the determination of copper (*Ibid.*, 25, 77), adopted as tentative last year, has been found, with slight modifications, to be satisfactory so far. This method is subject to interference by vanadium, chromium, and possibly a few other elements, but no definite proof has been found that these elements occur in fertilizers in sufficient quantity to affect results to any extent.

It has been found that an appreciable blank on the reagents is obtained in the titration by the short method. When this blank is subtracted from the titration of the sample the results check more closely with those obtained by the long method. The blank is 0.1–0.2 ml. of the sodium thio-sulfate solution. In determining the blank, and when the quantity of copper is small in the sample, it is best to add the starch solution before beginning the titration.

The long copper method may be necessary for greatest accuracy should it develop that there occurs an element that interferes in the short method. When zinc is also to be determined, it may be more convenient to use the long method. When only copper is to be determined, the short method is decidedly a time-saver. For these reasons, it is believed that both methods should be retained.

The gravimetric method for zinc (*This Journal*, 25, 361) and the volumetric method for zinc (*Ibid.*, 24, 309) as modified (*Ibid.*, 25, 78), both for samples containing 0.10 per cent or more of zinc, have continued to be satisfactory.

Work was done on the adaptation of the volumetric zinc method to the determination of less than 0.10 per cent of zinc. It was found best to use a quantity of sample containing at least 2.0 mg. of zinc. The chief difficulty was in the digestion of samples larger than 2 grams and in obtaining solutions that would not have enough color to interfere with the indicator color in the adjustment of the pH for the precipitation of zinc sulfide. In working on this problem, 10 gram samples were ashed at dull red heat, then treated according to the method except that more sulfuric acid was used. This produced solutions having less color than when the samples were only wet-ashed. Better results were obtained by the use of hydrogen peroxide in the digestion, even without previously dry-ashing the samples. It was also found that some zinc appeared to be lost when the insoluble residue was filtered out previous to the precipitation of the cupric sulfide. The method recommended is as follows:

#### TOTAL ZINC

##### *Volumetric Method*

(For samples containing less than 0.10% of zinc)

Weigh sufficient sample to contain at least 2.0 mg. of zinc, place in 300 ml. Erlenmeyer flask, add 10 ml. of  $\text{HNO}_3$ , and  $\text{H}_2\text{SO}_4$  equal to 6.0 ml. plus 0.5 ml. for

each gram of sample, and digest on hot plate to white fumes. Cool somewhat, add a little more  $\text{HNO}_3$ , digest again to white fumes, and repeat this treatment until the sample becomes no lighter. Cool somewhat, add a little more  $\text{HNO}_3$ , mix well, add a little 30%  $\text{H}_2\text{O}_2$ , mix well, digest to white fumes, and repeat this treatment until no further loss of color is obtained. Cool, add 100 ml. of water, mix well, and saturate with  $\text{H}_2\text{S}$ . Filter out  $\text{CuS}$  and other insoluble matter and proceed according to the volumetric method for the determination of zinc in samples containing at least 0.10% of zinc. ( $\text{CaSO}_4$ , which may remain on the filter with the  $\text{ZnS}$ , does not interfere.)

The colorimetric method for zinc (*This Journal*, 25, 78) was worked on collaboratively in 1940 and 1941 and found satisfactory. Since the volumetric method presented here for samples containing less than 0.10 per cent of zinc has been found successful, the colorimetric method has not been used on fertilizers in this laboratory, but the colorimetric method should be retained until collaborative work is done on the volumetric method for samples containing less than 0.10 per cent of zinc.

## REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS\*

By H. R. ALLEN, *Associate Referee*, and LELAH GAULT (Kentucky Agricultural Experiment Station, Lexington, Ky.)

In accordance with the recommendations of the Association (*This Journal*, 25, 48), collaborative studies were conducted on a method for elimination of neutralizing materials coarser than 20-mesh and on a method for electrometric titration. The substitution of a 0.5 *M* sodium carbonate solution for the molar solution now used was also investigated by collaboration.

### DIRECTIONS FOR COLLABORATIVE STUDY

A.—Mix Samples 1, 2, 3 thoroughly on glazed paper or oilcloth. Determine acid- or base-forming quality for Samples 1, 2, 3, and 4 by the tentative procedure, *Methods of Analysis*, A.O.A.C., 1940, 59, p. 37, using a 1 gram portion for Samples 1, 2, and 3 and a 0.25 gram portion for Sample 4. In the latter case, multiply result in ml. 0.5 *N*  $\text{NaOH}$  by 200. Omit corrections for acidity of nitrogen and of insoluble  $\text{P}_2\text{O}_5$ . After the titration, determine *pH* of the solution with the glass electrode apparatus if this apparatus is to be used for section C.

B.—Dissolve 53 grams of anhydrous  $\text{Na}_2\text{CO}_3$  or 143 grams of  $\text{Na}_2\text{CO}_3 \cdot 10 \text{ H}_2\text{O}$ , and 25 grams of sucrose, in water and dilute to 1 liter. Duplicate procedure A except for the *pH*, using 10 ml. of this normal  $\text{Na}_2\text{CO}_3$  solution and 20 ml. of normal  $\text{HCl}$ . Calculation is unchanged. Make a new blank titration.

C.—Apply the tentative method to Samples 1, 2, 3, and 4 as directed in A, but omit the filtering step before titration. Cool solution and titrate to *pH* 4.3, using glass electrode apparatus and a continuous stirrer. (The titration may be made in a 150 ml. beaker or the solution may be transferred to a larger beaker. A glass elec-

\* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

trode apparatus with a lock-down key is desirable for satisfactory work. A new blank titration must be made.)

D.—For Samples 1a, 2a, 3a, transfer entire sample (100 grams) to a 5 inch, 20-mesh sieve and separate by dry sieving. Place the portion coarser than 20-mesh in a 400 ml. beaker, add 100 ml. of water, and rotate several times. Let stand 5–10 minutes; transfer the sample to the 5 inch, 20-mesh sieve, which is supported by the walls of a 2 liter Pyrex beaker; and wash with a stream of distilled water from an overhead source to a volume of 1000 ml. as determined by a mark on the side of the beaker. Take care to direct the water stream uniformly on all parts of the sample. Place the sieve containing the washed, coarser-than-20-mesh portion on a watch-glass and dry in an oven at 70° to 75°C. for 4 hours. Cool, carefully remove all the sample from the sieve, and weigh. Prepare sample for analysis by grinding it to pass through a 0.5 mm. or 35-mesh sieve and mix well. Analyze by the tentative method as directed in A, using a 1 gram portion. Multiply result in pounds  $\text{CaCO}_3$  per ton by weight of the coarser-than-20-mesh portion divided by 100. (This is the amount of coarser-than-20-mesh material to be subtracted from the ash basicity of the whole sample before corrections are applied for nitrogen and insoluble  $\text{P}_2\text{O}_5$ .)

E.—In addition to the usual blank titration, make a blank determination, using the usual strength  $\text{Na}_2\text{CO}_3$  solution and omitting sample.

Report all results except for E in pounds of  $\text{CaCO}_3$  per ton.

### NOTES

It was decided to omit collaborative work with the Schroedter alkalimeter this year.

Samples 1a, 2a, and 3a are unground portions of Samples 1, 2, 3, respectively. To insure uniformity of the unground samples, the ingredients for these samples were weighed separately for each sample, to make a total of 100 grams. Additional portions of these samples will be sent upon request.

In the directions the basicity results of the original samples and of the coarser-than-20-mesh portions are not on the same moisture basis, but because of the fractions by which the latter results are multiplied, the discrepancy is believed to be negligible. It will be investigated further.

Sample 1 was approximately a 6–8–4 mixture. Samples 2 and 3 were 4–12–4 mixtures. Sample 4 was C.P. calcium carbonate. Samples 1, 2, and 3 were prepared according to the following formulas:

SAMPLE NUMBER	POUNDS PER TON		
Ammonium sulfate	293	176	176
Sodium nitrate	250	200	200
Sardine meal	200	—	—
Processed tankage	—	134	134
Superphosphate 20%	817	1200	1200
Muriate of potash	140	140	140
Dolomite, <20 mesh	150	100	—
Dolomite, >20 mesh	150	50	—
Sand, <20 mesh	—	—	100
Sand, >20 mesh	—	—	50



Sample 3 was the same composition as Sample 2 except that sand, free from carbonates, was substituted for dolomite. The coarser-than-20-mesh dolomite was washed on a 20-mesh screen with distilled water to eliminate all adhering finer particles, and dried. The dolomite had a basicity by the tentative method of approximately 2000 pounds calcium carbonate per ton.

Ground portions of Samples 1, 2, and 3 were sent to collaborators. Samples 1a, 2a, and 3a corresponded in composition to Samples 1, 2, and 3, respectively, but they were not ground. Collaborators' results are given in Table 1.

The following analysts collaborated in this work.

#### COLLABORATORS

1. H. R. Allen and Lelah Gault.
2. W. R. Austin and H. E. Witt, Armour Fertilizer Works, Nashville, Tenn.
3. C. R. Byers, Armour Fertilizer Works, Carteret, N. J.
4. R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
5. F. B. Carpenter, and H. L. Moxon, Virginia-Carolina Chemical Corporation, Richmond, Va.
6. T. L. Ogier, Agricultural Experiment Station, College Station, Tex.
7. H. L. Mitchell, Agricultural Experiment Station, Lafayette, Ind.
8. A. F. Spelman, Agricultural Experiment Station, Amherst, Mass.

#### DISCUSSION OF COLLABORATORS' RESULTS

Excellent checks were obtained for procedures A, B, and C by most of the collaborators. On the average, slightly lower results were found with the use of the 0.5 *M* sodium carbonate and of the glass electrode for titration than by the tentative method, but the differences were not significant. The study of the 0.5 *M* sodium carbonate was made to determine whether more nearly exact checks might be obtained by its use than with the stronger solution. Results were not conclusive and it seems advisable to study this point further. Results with the glass electrode warrant the adoption of this procedure as an optional tentative method. When many analyses are made much time is saved by the use of the electrometric titration, since the filtering step may be omitted.

The D result is the basicity of the coarser-than-20-mesh material, which is subtracted from the ash basicity of the whole sample if it is desired to eliminate basicity due to the coarser material. From the amounts of dolomite in the samples, the theoretical values are 150 pounds of calcium carbonate for Sample 1a, 50 pounds for Sample 2a, and none for Sample 3a. The average figures of the collaborators are 127 pounds for Sample 1a, 47 pounds for Sample 2a, and 1 pound for Sample 3a. The results were too low for Sample 1a. However, three analysts obtained results for this sample that were very close to the theoretical.

The differences in the weights obtained by the different collaborators for the washed, coarser-than-20-mesh portions were too large. In this labo-

ratory, three duplicate wet-sieved determinations were made on each of Samples 1a, 2a, and 3a. The average weights of the wet-sieved portions were 20.74, 22.84, and 22.98 grams, respectively. The maximum differences were 1.23 grams for Sample 1a, 1.13 grams for Sample 2a, and 2.18

TABLE 1.—*Collaborators' results*

COLLABORATOR	ASH BASICITY OR ACIDITY* (POUNDS CaCO <sub>3</sub> PER TON)					WEIGHT OF >20-MESH PORTION, GRAMS
	PROCEDURE A	PROCEDURE B	PROCEDURE C	PROCEDURE D†	PROCEDURES A MINUS D	
Samples 1 and 1a						
1	241	236	244	142	99	20.74
2	239	246	243	127	112	18.53
3	225	218	—	98	127	19.80
4	245	—	—	145	100	24.10
5	220	230	220	213‡	6‡	30.29‡
6	236	221	213	150	86	22.58
7	241	238	224	118	123	17.51
8	243	238	—	109	134	17.20
Average	236	232	229	127	112	20.07
Av. deviation	7	8	12	16	13	2.06
Samples 2 and 2a						
1	118	113	118	50	68	22.84
2	112	121	117	50	62	17.89
3	110	103	—	30	80	20.90
4	135	—	—	49	86	23.40
5	123	105	110	46	77	23.21
6	115	116	120	55	60	24.39
7	115	129	113	50	65	20.81
8	115	105	—	43	72	19.08
Average	118	113	116	47	71	21.56
Av. deviation	6	8	3	5	7	1.90
Samples 3 and 3a						
1	11A	19A	15A	1	12A	22.98
2	13A	2	17A	2	15A	13.89‡
3	20A	30A	—	2A	18A	20.10
4	0	—	—	2	2A	22.87
5	30A	30A	35A	2A	28A	22.78
6	24A	24A	23A	5	29A	22.82
7	18A	16A	28A	4	22A	19.41
8	20A	28A	—	1A	19A	19.10
Average	18A	24A	24A	1	18A	21.43
Av. deviation	6	8	6	2	6	1.63

TABLE 1.—Continued

COLLABORATOR	ASH BASICITY OR ACIDITY* (POUNDS CaCO <sub>3</sub> PER TON)					WEIGHT OF >20-MESH PORTION, GRAMS
	PROCEDURE A	PROCEDURE B	PROCEDURE C	PROCEDURE D†	PROCEDURES A MINUS D	
Sample 4						
1	2020	2010	2004	—	—	—
2	1936	1984	1940	—	—	—
3	2000	2000	—	—	—	—
4	1920	—	—	—	—	—
5	1985	2000	1980	—	—	—
6	1975	1970	1950	—	—	—
7	1978	1990	2003	—	—	—
8	2000	2010	—	—	—	—
Average	1977	1995	1975	—	—	—
Av. deviation	25	10	24	—	—	—
Theory	2000	2000	2000	—	—	—

\* Acidity results are followed by A. Other results are basicity.

† Theoretical values for procedure D, from coarser-than-20-mesh dolomite in the samples, are 150 pounds for Sample 1a, 50 pounds for 2a, and 0 for 3a.

‡ Omitted from average.

Procedure A.—Tentative method.

Procedure B.—Tentative method, except used 10 ml. 0.5 M Na<sub>2</sub>CO<sub>3</sub> and 20 ml. N HCl.

Procedure C.—Tentative method, except titrated without filtering, using glass electrode.

Procedure D.—Tentative method applied to coarser-than-20-mesh portions. Result in pounds CaCO<sub>3</sub> per ton is multiplied by weight of coarser-than-20-mesh portion divided by 100.

Procedures A minus D.—Result for Sample 1 minus result for Sample 1a, etc.

grams for Sample 3a. The differences in weights of the wet-sieved samples may be due to a number of circumstances, such as caking of the samples on standing, differences in moisture take-up while the relatively small, washed samples are prepared for analysis, and the difficulty in obtaining 100 gram unground samples in which the particle size aggregates of each material are the same in each sample for that material. To test the last theory, four 60 gram portions of the superphosphate used in preparing the collaborative samples were weighed and dry-sieved through the same 20-mesh screen. Weights of the finer-than-20-mesh portions were 31.75, 34.27, 34.18, and 33.34 grams, a maximum difference of 2.42 grams. In a further study of this problem, the Jones sampler riffle will be used in an effort to obtain more uniform samples. It should be recognized that this difficulty will always be present in sampling unground fertilizer samples.

The washed and dried coarser-than-20-mesh sample should be allowed to come to equilibrium with atmospheric conditions before its weight is taken, to reduce change in moisture content while this relatively small sample is prepared for analysis. To avoid making two moisture determinations, the original sample and its wet-sieved portion were not put on the same moisture basis as should be done for strict accuracy. In this laboratory, moisture determinations were made on the original samples

and on the washed coarser-than-20-mesh portions, after they had been dried at 75°C. The maximum difference in moisture content was 3 per cent. If the original sample and the coarser-than-20-mesh portion are placed on the same moisture basis, and if the coarser-than-20-mesh portion weighs 20 grams, this would make a maximum difference of plus 4.5 pounds calcium carbonate for Sample 1a, which contains 150 pounds of coarser-than-20-mesh dolomite per ton and a maximum difference of plus 1.5 pounds for Sample 2a, which contains 50 pounds of the coarser-than-20-mesh dolomite per ton.

The collaborative work and the experimental work conducted in this laboratory indicate that the procedure studied this year for eliminating neutralizing materials coarser than 20-mesh is the most practical one. The distillation method of Shollenberger<sup>1</sup> might be used, but the apparatus is difficult and requires reduced pressure. It would be impractical as a control method. Further study of the two procedures reported by the Associate Referee last year (*This Journal*, 25, 345) indicates that they also are not suitable for control methods.

In the present tentative method the blank is determined by direct titration. In this laboratory lower results have sometimes been obtained if a blank determination was made. The collaborators were asked to report both a blank titration and a blank determination. The maximum difference reported in the two was 0.1 ml. of 0.5 *N* sodium hydroxide, which is not enough for a recommended change in procedure. Collaborators reporting on procedure C, with the glass electrode, were asked to determine the pH of the indicator-titrated solution obtained by procedure A. The values reported, 4.25, 4.3, 4.3, 4.52, 4.26, 4.35, and 4.47, indicate that little trouble was experienced in obtaining the correct end point when the mixed indicator was used for titration.

#### ADDITIONAL EXPERIMENTAL DATA

In connection with the study of the 0.5 *M* sodium carbonate solution it seemed advisable to determine whether the weaker sodium carbonate would retain all the chlorides and phosphates in the sample at the ignition temperature. The chloride and phosphate contents of Samples 1 and 2 and of 9-18-18 mixture, No. 4340, were determined; 1 gram portions of Samples 1 and 2 and 0.5 gram portions of Sample 4340 were evaporated with 10 ml. of *M* sodium carbonate for one group and with 10 ml. of 0.5 *M* sodium carbonate for another group, and were ashed at 600°C. in the usual manner. The chloride and phosphate contents of the ashed samples were determined. Results showed there was no loss of chloride or phosphate when the weaker sodium carbonate was used. Results are given in Table 2.

The use of a short, solid, glass rod to remove the ashed material from

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<sup>1</sup> *Soil Science*, 30, 301 (1930).

TABLE 2.—Comparison of  $M$   $\text{Na}_2\text{CO}_3$  and  $0.5 M$   $\text{Na}_2\text{CO}_3$  in retaining chloride and phosphate at  $600^\circ\text{C}$ .  
(Results in per cent)

SAMPLE NUMBER	SAMPLES NOT ASHED		SAMPLES ASHED			
			Cl		$\text{P}_2\text{O}_5$	
	Cl	$\text{P}_2\text{O}_5$	$M$ $\text{Na}_2\text{CO}_3$	$0.5 M$ $\text{Na}_2\text{CO}_3$	$M$ $\text{Na}_2\text{CO}_3$	$0.5 M$ $\text{Na}_2\text{CO}_3$
1	3.65	8.95	3.85	3.75	9.05	9.25
2	3.65	12.40	3.75	3.75	12.65	12.60
4340	14.10	16.50	14.70	14.40	16.70	16.50

the bottom of the beaker before the addition of the 30 ml. of  $N$  hydrochloric acid was tried. The rod was left in the beaker during the digestion period, and it was used to decant the solution in filtering. The rod was not long enough to interfere with the watch-glass over the beaker. As a rule, somewhat higher basicity results were obtained by this procedure.

#### SUMMARY

Collaborative analyses were conducted on—

- (1) The tentative method.
- (2) Substitution of  $0.5 M$  sodium carbonate solution.
- (3) Titration with the glass electrode.
- (4) Elimination of neutralizing materials coarser than 20-mesh.

Uniformly good results were obtained by the collaborators except on some of the analyses on Samples 1a of D section and on the weights of the wet-sieved, coarser-than-20-mesh portions.

The optional method follows:

Proceed as directed in 59, pp. 37–38, *Methods of Analysis, A.O.A.C.*, through the addition of 50 ml. of water and 30 ml. of normal  $\text{HCl}$  and digestion on hot plate or steam bath for 1 hour. Cool to room temperature, and without filtering titrate the solution in the 150 ml. beaker to  $\text{pH}$  4.3, using a glass electrode apparatus or other standard means of electrometric titration and a continuous stirrer. A new blank titration should be made. Calculate results as directed in 59, p. 37.

#### REPORT ON CRUDE FAT OR ETHER EXTRACT IN OIL-BEARING MEALS

By J. J. TAYLOR (Department of Agriculture, Tallahassee, Fla.),  
*Associate Referee*

The reason for undertaking this comparative study was the receipt by Dr. W. W. Skinner of a request by a member of the Finished Materials

Standards Committee of the National Soybean Processors Association that the Association of Official Agricultural Chemists consider giving official recognition to the A.O.C.S. method for determining fat (oil) in oil meals as an alternative method, and that this method be published in the book of methods of the A.O.A.C.

It was pointed out that A.O.A.C. methods had been adopted as the official methods of the N.S.P.A. with the single exception that the American Oil Chemists Society method for determining fat had been adopted as official instead of the A.O.A.C. for oil-bearing meals.

The objective of the study was to determine whether or not the two methods, used on several different oil-bearing meals by different analysts, would give results sufficiently close to justify a recommendation that the A.O.C.S. method be added to the present book of methods as an alternative method.

Twenty collaborators participated in the work, some belonging to the A.O.A.C. group and some to the A.O.C.S. group. A tabulation of results, with the number designating the participant, is given.

The materials used were (1) soybean oil meal, (2) cottonseed meal, (3) peanut oil meal, and (4) linseed oil meal. Instructions to collaborators specified at least three determinations by both the A.O.A.C. and the A.O.C.S. methods, and a report of the average on each of the four samples. The Butt type extractor and the Goldfish extractor were the principal ones used, although other extractors were used in some instances.

Soon after work was begun, one collaborator reported that in using the Goldfish extractor for the A.O.C.S. method, the petroleum benzin did not percolate through the alundum thimble fast enough to carry off the condensate, resulting in an accumulation of liquid and slowing down of the extraction. Therefore collaborators using this type of extractor were requested to run some additional determinations and to continue the extraction for five hours instead of the three hours called for by the method. These results are not included in the tabulation, but they indicate that the precaution was not necessary as the additional amount of oil resulting from the five-hour extraction was, in most cases, insignificant.

It will be noted that most collaborators report higher results for the A.O.A.C. method than for the A.O.C.S. method. This is true for all four samples. The average difference for each sample is as follows: No. 1, .25 per cent; No. 2, .26 per cent; No. 3, .21 per cent, and No. 4, .29 per cent. The average difference for all samples is .25 per cent higher for the A.O.A.C. method.

This study was not intended to establish the relative merits of the two methods, nor is it intended to comment on them here. The only purpose was to establish whether or not results obtained by different collaborators, using both methods checked closely enough to justify making both methods official for determining fat on this type of material.

*Results of comparative study of A.O.A.C. and A.O.C.S. methods for fat in oil-bearing meals*

COLL. NO.	SAMPLE NO. 1 SOYBEAN OIL MEAL			SAMPLE NO. 2 COTTONSEED MEAL			SAMPLE NO. 3 PEANUT OIL MEAL			SAMPLE NO. 4 LINSEED OIL MEAL		
	A.O.C.S.	A.O.A.C.	DIFF.	A.O.C.S.	A.O.A.C.	DIFF.	A.O.C.S.	A.O.A.C.	DIFF.	A.O.C.S.	A.O.A.C.	DIFF.
1	3.80	4.09	0.29	5.36	5.77	0.41	5.76	5.98	0.22	4.45	4.99	0.54
2	4.26	4.40	0.14	6.16	6.40	0.24	5.79	6.24	0.45	4.86	5.18	0.32
3	3.84	4.02	0.18	5.23	5.91	0.68	5.26	5.74	0.48	4.53	4.74	0.21
4	3.70	3.75	0.05	5.28	5.28	0.00	5.64	5.75	0.11	4.32	4.45	0.13
5	3.83	4.45	0.62	5.69	6.47	0.78	5.42	6.10	0.68	4.38	4.90	0.52
6	3.83	4.17	0.34	5.54	6.40	0.86	5.87	6.21	0.34	4.52	5.11	0.59
7	3.75	3.83	0.08	5.28	5.53	0.25	5.69	5.77	0.08	4.42	4.38	0.04*
8	3.88	4.03	0.15	5.22	5.60	0.38	5.70	5.75	0.05	4.58	4.82	0.24
9	3.85	4.02	0.17	5.57	5.35	0.22*	5.88	5.88	0.00	4.57	4.75	0.18
10	3.82	3.85	0.03	5.27	5.63	0.36	5.68	5.94	0.26	4.38	4.64	0.26
11	3.75	3.87	0.12	5.28	5.25	0.03*	5.72	5.65	0.07*	4.38	4.65	0.27
12	3.67	3.95	0.28	5.23	5.09	0.14*	5.65	5.49	0.16*	5.36	4.66	0.70*
13	3.80	3.76	0.04*	5.37	5.58	0.21	5.70	5.79	0.09	4.41	4.55	0.14
14	3.87	4.07	0.20	5.42	5.78	0.36	5.80	6.08	0.28	4.55	4.97	0.42
15	3.88	4.01	0.13	5.82	5.74	0.08*	5.90	5.79	0.11*	4.76	4.77	0.01
16	4.25	4.35	0.10	5.82	5.92	0.10	6.42	7.16	0.74	5.12	5.92	0.80
17	3.80	4.42	0.62	5.32	5.41	0.09	5.70	5.94	0.24	4.39	4.53	0.14
18	3.74	4.58	0.84	5.29	5.52	0.23	5.76	6.02	0.26	4.40	5.16	0.76
19	3.74	4.13	0.39	5.21	5.65	0.44	5.56	5.87	0.31	4.35	4.92	0.57
20	3.79	4.05	0.26	5.36	5.75	0.39	5.75	5.80	0.05	4.43	4.95	0.52
A.	3.84	4.09	0.25	5.44	5.70	0.26	5.74	5.95	0.21	4.56	4.85	0.29

\* Indicates loss by A.O.A.C. method.

Collaborators showed keen interest in the project, many of them doing additional work not called for in the project but bearing on the general subject of fat extraction. One collaborator comments as follows: "It is my belief that the routine determination of oil by solvent extraction merits thorough investigation." This comment seems justified in view of the wide discrepancies found among collaborators in this study.

One of the factors contributing to these discrepancies is probably a lack of uniform procedure with respect to moisture under the A.O.C.S. method, particularly in the case of soybean oil meal.

One collaborator comments that "ethyl ether extraction gives yields ranging from .24 to .51 per cent more lipid material than petroleum ether." He also raises the question as to whether all of this lipid material may be considered as "oil." Probably these and other points should be made the subject of more thorough study.

The results of this study indicate that the official recognition of the A.O.C.S. method at this time would lead to confusion since there is a consistent difference in results obtained by the use of the two methods, amounting in many cases to more than any tolerance allowed under regulatory laws.

## REPORT ON CAROTENE IN FEEDING STUFFS

By A. R. KEMMERER (Agriculture Experiment Station, College Station, Texas), *Associate Referee*

Last year the recommendations of the Associate Referee were adopted by this Association, *This Journal*, **25**, 46.

In the work this year the collaborators were asked to analyze two samples: No. 1, alfalfa meal and No. 2, yellow corn. The yellow corn was analyzed for carotene and cryptoxanthin by the complete and abridged chromatographic methods studied last year (*Ibid.*, 886). The alfalfa meal was analyzed for pure carotene by two procedures, the adsorptive technique employing activated magnesium carbonate, tentatively adopted last year, and a modification of the abridged chromatographic method, which is given later in the recommendations in this report.

The Associate Referee appreciates the generous cooperation of the following collaborators:

C. J. Watson, Department of Agriculture, Ottawa, Canada.

J. W. Kuzmeski, Massachusetts Agr. Exp. Station, Amherst, Mass.

B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.

P. W. Punnett, M. E. Bader, and L. W. Hartzel, Good Housekeeping, New York.

F. E. Randall, Cooperative G. L. F. Mills, Buffalo, N. Y.

J. C. Fritz, Borden, Elgin, Ill.

L. Kishlar, B. Beaver, and Wm. Brew, Purina Mills, St. Louis, Mo.



## COMMENTS OF COLLABORATORS

*C. J. Watson.*—If a number of determinations are to be made, it is more convenient to use an aliquot for removal of xanthophyl with methanol instead of the whole of the skellysolve solution.

*B. L. Oser.*—The carotene analyses of both the alfalfa and yellow corn were conducted by the complete as well as abridged chromatographic methods. Some difficulty was experienced in the preparation of a magnesium oxide column which would permit the formation of sharp, well defined bands. This difficulty was partially eliminated by packing the column evenly, but not too tightly, and allowing the extract to be drawn through rather rapidly.

*P. W. Punnett.*—In spite of the fact that the column was packed in several ways, sharp zones were never obtained. Band delineation was best in a very tightly packed column, but this increased the separation time and the difficulty of band removal from the column.

## EXPERIMENTAL

Because information is needed on the methods most widely used for pure carotene and because it is difficult to make a complete collaborative

TABLE 1.—*Comparison of methods for pure carotene*

LABOR- ATORY NUM- BER	PRODUCT	CRUDE CARO- TENE	CAROTENE X—REAGENT		CAROTENE DIACETONE		PURE CAROTENE ABRIDGED	PURE CAROTENE
			IMPU- RITY		IMPU- RITY			CALC. FROM CAROTENE X—REAGENT
		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>per cent</i>	<i>p.p.m.</i>	<i>per cent</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
65667	Alfalfa	30.0	24.2	10.3	25.2	9.1	24.6	21.6
65668	Alfalfa	106.4	92.0	5.5	96.0	6.9	90.4	86.9
59718	Alfalfa—A.O.A.C. Sample No. 1	101.2	82.4	4.3	83.2	5.2	74.4	78.8
65915	Alfalfa—A.O.A.C. Sample No. 2	35.6	30.4	5.5	31.6	12.2	27.2	28.7
65784	Canned Spinach—old	44.0	38.0	9.5	40.4	15.5	34.8	34.4
65914	Canned Pumpkin	62.9	62.9	4.2	65.1	5.4	57.1	60.3

study on a number of samples, some work done by the Associate Referee is included in this report. Pure carotene was determined in samples by the two methods sent out this year, and by the method of Hegsted, Porter, and Peterson,<sup>1</sup> which involves the use of dilute diacetone (6 volumes of water plus 100 volumes of diacetone). The pure carotene fractions obtained by the diacetone treatment and by shaking with activated magnesium carbonate were subjected to analysis by the complete chromatographic method in order to ascertain the amounts of impurity they contained. The data from this work are shown in Table 1. It is evident that neither the treatment with activated magnesium carbonate nor that with diacetone removes all the impurities, but that the former gives a more nearly pure solution than the latter. The abridged chromatographic

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 11, 256 (1939).

method gives a very pure solution. The pure carotene (p.p.m.) obtained by this method compares very favorably with that calculated from the constituents of the so-called pure carotene obtained by the treatment with activated magnesium carbonate.

#### DISCUSSIONS OF COLLABORATIVE RESULTS

In Table 2 are shown the results of the collaborative study on the two methods sent out for pure carotene in the alfalfa sample. In this work the results obtained by the abridged chromatographic procedure are only slightly lower than those obtained by shaking the crude carotene solution with activated magnesium carbonate. Considering the data in Tables 1 and 2, the Associate Referee believes that either the abridged chromato-

TABLE 2.—*Crude and pure carotene in alfalfa sample as reported by collaborators*

ANALYST NO.	CRUDE CAROTENE	PURE CAROTENE X—REAGENT	PURE CAROTENE ABRIDGED CHROMATOGRAPHIC
	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
1	40.8	37.1	33.9
2	52.0	33.0	36.0
3	37.3		32.1
4	89.0*	61.0*	
5	39.7	37.2	36.3
6	41.0	35.8	35.0
7	42.0	35.4	35.8
8	35.2	30.4	27.6
Mean	41.0	34.8	33.8

\* Omitted from mean.

graphic or the activated magnesium carbonate procedure is better for the determination of pure carotene than are methods involving the use of diacetone. The treatment with activated magnesium carbonate is limited to dried hays and plants, and it is not suitable for materials containing lycopene or cryptoxanthin. If a high degree of purity is desired, the abridged chromatographic method should be used.

In Table 3 are given the results of the collaborative study on the complete chromatographic method for yellow corn. Five collaborators reported their results. The agreement between the collaborators on the parts per million of cryptoxanthin is as good as can be expected, and three of the five collaborators obtained good agreement for the beta carotene. The agreement for alpha carotene is fair, but for K carotene the results are wide. The K carotene band is very close to the beta carotene band, and it is very difficult to separate these two pigments clearly. Considering the results obtained this year and last year the Associate Referee is of the opinion that the complete chromatographic method

TABLE 3.—*Results of collaborative study on complete chromatographic method for yellow corn*

ANALYST NUMBER	CRUDE CAROTENE	IMPURITY	CRYPTO- XANTHIN	K CAROTENE	BETA CAROTENE	ALPHA CAROTENE	RECOVERY
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	per cent
3	5.20	.16	2.91	.32	1.44	.32	99.0
4	5.28		2.50		1.04	.12	69.3
5	5.42	.42	2.83	.67	.78	.20	90.4
7	4.64	.18	2.19	.06	.40	.09	61.0
8	5.45	.38	2.87	.17	1.63	.14	95.2

should be further studied. When the purity of a carotene solution or the constituents of the crude carotene fraction from any material are desired a complete chromatographic method is needed. For this method the selection of an adsorbent with the proper qualifications is important. However, if an adsorbent is too retentive and the pigments are retained at the top of the column, with little or no separation of bands, the addition of a more polar solvent such as benzol (25 per cent) to the petroleum benzin will cause the bands to separate. The benzol can be removed from the column by subsequent washing with petroleum benzin.

In Table 4 are given the results obtained by the abridged chromatographic method. Three of the five collaborators obtained results that agreed well. This method is rapid and simple to operate, and it affords a good means of separating carotene, cryptoxanthin, and impurities in a crude carotene solution. It is not suitable for the separation of the alpha, beta, gamma and K carotene.

The methods for carotene that have been adopted or tested by this Association are limited to crude and pure carotene in dried hays and other dried plants and in yellow corn, but it is well that methods for other materials be referred to here in order that they can be tested and acted on in the future. The methods for other materials usually involve only slight modifications of the original method. For a number of materials the method of preparation of the sample is important. In fresh green materials the carotene is stabilized by placing the material in alcohol, This

TABLE 4.—*Results of collaborative study on the abridged chromatographic method for yellow corn*

ANALYST NUMBER	CRUDE CAROTENE	CRYPTOXANTHIN	CAROTENE	IMPURITY
	p.p.m.	p.p.m.	p.p.m.	p.p.m.
3	5.5		4.5	
4	5.7	1.5	1.4	
5	5.3	2.6	2.3	.22
7	4.5	2.4	1.9	.22
8	5.0	2.7	2.0	.30

*Journal*, 24, 743. For canned goods the liquid is drained off, and the solids are weighed and finally ground in a food chopper. For dehydrated fruits or vegetables that are high in sugar, such as dried apricots, carrots, or sweet potatoes, the weighed sample is soaked overnight in about 10 volumes of water. A Waring blender is helpful in the extraction of these samples. Dehydrated leafy vegetables need no special preparation. Samples of butter are prepared by heating in an electric oven at 60°C. and allowing the casein and water to settle. The clear liquid fat is poured into a fluted filter and the sample is allowed to filter. Samples of body fats are heated at 90°C. and filtered.

Some materials require slightly modified chemical procedures. Sweet potatoes are refluxed with 95 per cent ethanol instead of alcoholic potash, *Ibid.*, 744. Tomatoes, apricots, and watermelons, which contain considerable lycopene, require the use of the abridged chromatographic method as given in the recommendations. The complete chromatographic method is required for carotene and cryptoxanthin in egg yolk, but the amount of these pigments is usually so low that determination of the crude carotene suffices. For rat excrement or other fecal material the complete chromatographic method is used with a magnesium oxide of high adsorptive power. For commercial crystalline carotene the complete chromatographic method is needed.<sup>2</sup> For blood plasma, or serum, the samples are refluxed for 15 minutes instead of 30 minutes.

## REPORT ON RIBOFLAVIN IN FEEDING STUFFS

By A. R. KEMMERER (Agricultural Experiment Station, College Station, Texas), *Associate Referee*

The microbiological method of Snell and Strong (1) and the fluorometric method of Hodson and Norris (2) have been studied collaboratively for riboflavin in dried skim milk, yeast, dehydrated alfalfa meal, whole wheat, and white flour (3, 4). Both methods worked well for the yeast, dried milk, and alfalfa, but they did not give reliable results for the flours. In the discussion of the microbiological method in last year's report (4) it was pointed out that the flours might contain materials that either enhanced or inhibited the acid production of *Lactobacillus casei*. For the last two years a number of workers have carried out experimental work with the idea of eliminating these factors. Andrews, Boyd, and Terry (5, 6) and Scott, Randall, and Hessel (7) reported that treatment of flour extracts with takadiastase removed to a large extent the factors that affected the assay. On the other hand Wright (8) and Wegner, Kemmerer, and Fraps (9) found digestion with takadiastase to give no

<sup>2</sup> Fraps and Kemmerer, *Ind. Eng. Chem., News Ed.*, 19, 840 (1941).

improvement. Wright (8) supplemented the basal medium with xylose, asparagin, nicotinic acid, and pantothenic acid and obtained some improvement. However at low assay levels high results were still obtained. Wegner et al. (9) found that extracts of rice bran, wheat bran, and whole wheat flour, which had been freed of riboflavin, stimulated acid production when added to known amounts of riboflavin. This stimulation was greater at low levels of riboflavin than at high levels. Addition of these extracts to the basal medium did not improve the method. Clarke, Lechycska, and Light (10) found that in the pantothenic assay rice polish extract stimulated the production of acid by *Lactobacillus casei*. Bauernfeind, Sotier, and Boruff (11) reported that fatty acids stimulated the bacteria and that the method could be improved by extracting the finely ground samples with ether. Strong and Carpenter (12) reported that interfering substances could be removed by adjusting the acid extracts of samples to pH of 4.5, filtering, and then shaking the filtrate with ether. With some samples filtration at pH 4.5 was sufficient. Wegner, Kemmerer, and Fraps (13) found that precipitation at 4.5 alone removed the impurities and greatly improved the method. The precipitate did not contain riboflavin but enhanced the action of added riboflavin. Extraction of the finely ground material with ether improved the results somewhat but not to as great an extent as did the precipitation at 4.5. Landy and Dicken (14) devised a medium in which all the constituents, except casein hydrolysate, were chemically defined. With this medium they report that they were able to successfully determine riboflavin, pantothenic acid, pyridoxin, nicotinic acid, folic acid, and biotin.

The fluorometric methods have likewise been modified in attempts to obtain more nearly accurate results. Conner and Straub (15) employed adsorption with supersorb, followed by oxidation with potassium permanganate and hydrogen peroxide. These authors applied their technic to the determination of riboflavin in wheat and corn (16). Swaminathan (17) adsorbed the riboflavin from acid extracts on fullers earth, eluted the riboflavin, and then destroyed the riboflavin in an aliquot of the eluate by heating with dilute sodium hydroxide. Both aliquots of the eluate were purified by oxidation with permanganate and hydrogen peroxide. The fluorescence of both solutions was measured and the riboflavin determined by difference. For animal tissues Van Duyne (18) digested the fresh ground material with 0.3 per cent pepsin in 0.2 per cent hydrochloric acid to destroy fluorescing material that interfered. The fluorescence was measured directly without further treatment.

An entirely new technic, which involves polarographic measurements, has been developed by Lingane and Davis (19) for pure riboflavin in solution and for riboflavin in hydrochloric acid extracts of yeast.

The collaborators this year were asked to analyze two samples, No. 1, whole wheat flour, and No. 2, white flour, by a modified microbiological

procedure, which is given later in the recommendations, or by any other method with which they had had experience. The two samples were the same as those sent out last year (4).

### COLLABORATORS

The generous cooperation of the following collaborators is greatly appreciated:

- J. W. Kuzmeski, Massachusetts Agr. Exp. Sta., Amherst, Mass.  
L. C. Norris, Cornell University, Ithaca, N. Y.  
J. E. McConkie and W. Popper, Jr., California Packing Corp., San Francisco, Calif.  
B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.  
H. J. Prebluda, U. S. Industrial Chemicals, New York  
G. O. Cragwall and co-workers, Chas. Pfizer and Co., Inc., New York  
W. J. Rudy, Allied Mills, Inc., Peoria, Ill.  
I. Olcott, Dawe's Products Company, Chicago, Ill.  
H. F. Seibert and M. Bicking, S.M.A. Corporation, Chagrin Falls, Ohio  
F. E. Randall, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.  
J. C. Fritz and C. L. Hart, Bordens, Elgin, Ill.  
M. Rosenblatt, Schenley Distillers Corporation, New York  
C. O. Gourley, The Beacon Milling Company, Inc., Cayuga, N. Y.  
E. B. Vliet and E. Willerton, Abbott Laboratories, N. Chicago, Ill.  
K. G. Falk, Vitamin Testing Laboratory, New York  
O. I. Struve, Eastern States Coop. Mill. Corp., Buffalo, N. Y.  
L. Kishlar, Purina Mills, St. Louis, Mo.  
F. W. Tanner, Jr., New York Agricultural Experiment Station, Geneva, N. Y.

### SUMMARY OF COMMENTS OF COLLABORATORS

*J. W. Kuzmeski.*—The riboflavin was determined according to the fluorometric method tentatively adopted (3). The results are given in Table 1, Collaborator 1.

*L. C. Norris.*—Unsatisfactory results were obtained by the procedure sent out. A slightly modified technic gave good results. After the acid extracts had been filtered at a pH of 4.5, the whole wheat-flour extract was adjusted to a pH of 5.7 and the white flour to a pH of 6.0 (i.e., the pH just below the point at which turbidity begins). The extracts were then filtered and assayed. The samples were also assayed by the procedure of Scott, Randall, and Hessel (7), which involves digestion of water extracts with takadiastase and filtration and by the fluorometric method tentatively adopted (1). The results are given in Table 1, Collaborator 2.

*J. E. McConkie and W. Popper, Jr.*—A number of variations in procedure were tried. The data obtained indicate lower and more consistent results from papain digestion than from autoclaving alone. However, the introduction of minor variations in manipulative detail tended to invalidate any definite conclusions.

*B. L. Oser.*—The samples were analyzed by a procedure involving digestion with takadiastase. The results obtained were practically the same as with the collaborative procedure (Table 1, Collaborator 4).

*G. O. Cragwall and Co-workers.*—The samples were assayed by the A.O.A.C. procedure tentatively adopted for yeast and dried skim milk (3) and by modified procedures, involving trituration with sand and digestion with takadiastase. The results are given in Table 1 (Collaborator 6).

*I. Olcott.*—It is recommended that after the samples have been autoclaved they be cooled thoroughly, agitated, and then autoclaved a second time.

TABLE 1.—Results of collaborative study on riboflavin (*p. p. m.*)

ANALYST	WHOLE WHEAT FLOUR			WHITE FLOUR		
	REFERENCE BACTERIOLOGICAL METHOD	OTHER BACTERIOLOGICAL METHODS	FLUOROMETRIC METHOD	REFERENCE BACTERIOLOGICAL METHOD	OTHER BACTERIOLOGICAL METHODS	FLUOROMETRIC METHOD
1			.69			.38
2		1.07	1.00*		.61	.51*
3	.95	.60*	.54*	.31		.54
4	.74	.76*		.34	.27*	
5	.77			.31		
6		1.02	.96*		.50	.45*
7	1.26†		1.20, 1.45, 2.00	.35		.43
8	.84			.66†	.74	
9	1.05	1.06		.34	.38	
10	.96	1.23*	1.06	.37	.53*	.53
11	1.02			.57†		
12	.94			.61†		
13	.99			.49		
14	.95	.71*		.38		
15	.91			.41		
16	.87		.49	.36		.29
17	.52†	.72*		.23†	.33*	
18	.99	.69		.38		
19	.84			.34		
Mean	.91	.88	1.13	.41	.48	.43
Mean corrected	.92			.38		

\* Enzyme digestion.

† Omitted from corrected mean.

*H. F. Seibert and M. Bicking.*—The samples were also assayed by the method of Landy and Dicken (14). Both methods gave accurate and reproducible results but the method of Landy and Dicken is preferred because it has been found easier to handle.

*F. E. Randall.*—Extraction of samples with water and digestion with takadiastase is preferable. This procedure invariably gives higher results in most materials. The samples were also assayed by a fluorometric procedure (Table 1, Collaborator 10).

*James C. Fritz and C. L. Hart.*—The values obtained seem unusually low for wheat flour. However, a number of other samples run simultaneously on the same assay gave results fully as high as expected.

*C. O. Gourley.*—Trouble was experienced in the filtration of the samples.

*E. B. Vliet and E. Willerton.*—The results with the whole wheat flour show evidence of some interfering factor operating at levels of 2.5 ml. or above. Takadiastase digestion was made in hope of eliminating part of the difficulty, but without success.

*O. I. Struve.*—The samples were also run by the fluorometric method of Conner and Straub (15), and considerably lower values were obtained than by the collaborative method (Table 1, Collaborator 16).

*L. Kishlar and H. Brewer.*—The results obtained by the collaborative procedure were somewhat lower than those usually obtained for flours. Somewhat higher results were obtained by a procedure involving digestion with takadiastase (Table 1, Collaborator 17).

*F. W. Tanner, Jr.*—The whole wheat flour was also run by a modified procedure, and a more sensitive strain of *Lactobacillus casei* was used (Table 1, Collaborator 18).

## DISCUSSION OF RESULTS

The results obtained by the various collaborators are shown in Table 1. The mean (corrected) riboflavin content of the whole wheat flour is 0.92 parts per million and the maximum variation from the mean is 43.5 per cent; 14 of the 16 collaborators obtained results within 20 per cent of the mean. The average (corrected) riboflavin content of the white flour is 0.38 part per million and the maximum variation from the mean is 73.6 per cent; eleven of 16 collaborators obtained results within 20 per cent of the mean. The samples sent out are the same ones that were analyzed last year (4) by the microbiological method tentatively adopted for yeast and dried skim milk. The results with this method were 1.45 parts per million for the whole wheat flour and 0.65 part per million for the white flour.

Some of the collaborators made the analyses by modified bacteriological and by fluorometric methods, and these results are also given in Table 1. One of the principal modifications in the microbiological method was digestion with takadiastase. In most cases no better results were obtained than were obtained with hydrochloric acid hydrolysis. The main factor that improves the results is the filtration at pH of 4.5. One of the collaborators ran the samples by the Landy-Dicken (14) procedure and obtained results that agreed with the results obtained by the collaborative method.

The results from this study show that the modified method is an im-



TABLE 2.—*Variation between the various levels of samples, p.p.m. riboflavin*

ANALYST NO.	WHOLE WHEAT FLOUR				WHITE FLOUR			
	1	2	3	4	2	3	4	5
	ml.	ml.	ml.	ml.	ml.	ml.	ml.	ml.
3	.62	1.02	.96	.88	.28	.31	.33	.33
5		.92	.80	.79	.80	.67	.64	.53
9	.94	1.02	1.12	1.12	.38	.36	.38	.39
10	.96	.99	.89	.99	.36	.37	.37	.37
11	1.04	.90	1.07	1.05	.62	.57	.55	.57
12	1.26	.90	.82	.77	.58	.68	.61	.58
13	1.40	1.03	.96	.98	.67	.52	.45	.47
14	1.00	1.03	.88	.82	.38	.39	.39	.37
17	.50	.48	.54	.57	.30	.18	.22	.23
18					.40	.36	.32	.28
19		.90	.84	.77		.35	.33	.34

provement over previous methods and that as reliable results as can be expected are obtained. The agreement between assay levels is also fairly good, as is shown in Table 2. The greatest discrepancy is in the 1 ml. level of whole wheat flour and the 2 ml. level of white flour. However, these levels should not have much weight in the deductions, as in most cases they contained such low amounts of riboflavin that they are below the range of the greatest accuracy of the method.

The results obtained this year by the modified method were much lower than those obtained by the method used last year. These lowered results, as might be supposed, are not due to adsorption or occlusion of riboflavin on the precipitate that forms at pH of 4.5. Precipitates from a large number of different kinds of materials have been tested in this laboratory (13) and found to contain no detectable amounts of riboflavin.

The tentative microbiological method for the determination of riboflavin in yeast and dried skim milk, modified for wheat flours or other materials containing less than 10 parts per million of riboflavin, follows:

To 10 grams of whole wheat or white flour add ca. 140 ml. of 0.1 N HCl. Mix well and autoclave 15 minutes at 15 lbs. pressure. Cool to room temperature. By means of a pH apparatus adjust the pH to 4.5 with 1 N NaOH. Filter the sample carefully, pouring the first few ml. that come through back onto the filter paper. Wash the precipitate two times with 10 ml. portions of distilled water buffered with 1 ml. of phosphate solution, *This Journal*, 24, 417(e), and adjusted to pH of 4.5 with 0.1 N HCl. Adjust the pH of the filtrate to 6.6–6.8 and dilute to 200 ml. Assay the solutions at 2 or more levels, such as 2 and 3 ml. for whole wheat flour and 3 and 4 ml. for white flour. Put 4 tubes on each level. Set up standard tubes (4 on each level) on 0.0, 0.05, 0.10, 0.15, and 0.20 microgram levels of riboflavin.

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## REPORT ON SOLUBLE CHLORINE IN FEEDING STUFFS\*

By JOHN W. KUZMESKI (Massachusetts Agricultural Experiment Station, Amherst, Mass.), *Associate Referee*

The Associate Referee submitted four samples of mixed feed to seven collaborators for determination of soluble chlorine by substantially the method reported last year (*This Journal*, **26**, 870).

Methods have been accepted by this Association as official for the standardization of the silver nitrate and as tentative for potassium sulfocyanate solutions used in the proposed method for soluble chlorine (*This Journal*, **24**, 631). Also, the possibility of error due to difficulty in obtaining a representative analytical sample from a feed mixture containing a high percentage of chlorine will be present in any method that may be adopted for the determination of this element. Since these two factors are outside the scope of this investigation it was decided to eliminate them as possible contributors toward any lack of agreement in the results reported by the several collaborators.

Therefore portions of the same silver nitrate and potassium sulfocyanate solutions standardized by the Associate Referee and composite samples of dairy and poultry feeds were sent to each collaborator. Sample A, consisting of 2.8 grams of composite dairy feed plus 0.2 gram of potas-

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\* Contribution 458 of the Massachusetts Agricultural Experiment Station.

sium chloride, and Sample B, consisting of 2.7 grams of composite poultry feed plus 0.3 gram of potassium chloride, were sent in triplicate as accurately weighed 3 gram charges.

The collaborators were asked to use 12 ml. of the silver nitrate solution and 1 gram aliquots for the composite dairy and poultry feeds, and 35 ml. of silver nitrate solution and 0.5 gram aliquots for Samples A and B. The recommended volumes of silver nitrate solution were considerably more in excess of the theoretical volumes required than the 10 per cent excess formerly recommended. It was thought the method would be more adaptable to routine analysis if it could be shown that a large excess of silver-nitrate solution would not affect adversely the complete recovery of added chlorine in Samples A and B.

### METHOD

#### REAGENTS

(a) *Potassium chloride solution*.—Recrystallize reagent KCl three times from water, dry at 110°C., then heat at ca. 500°C. to constant weight. Dissolve 2.1026 grams in distilled water and dilute to 1 liter. Solution contains 0.001 gram of Cl per ml.

(b) *Silver nitrate solution*.—Dissolve 5 grams of  $\text{AgNO}_3$  in 1 liter of distilled water and adjust solution so that 1 ml. equals 1 ml. of standard KCl solution.

(c) *Potassium sulfocyanate solution*.—Dissolve 2.5 grams of KSCN in 1 liter of distilled water and adjust so that 1 ml. equals 1 ml. of standard  $\text{AgNO}_3$  solution. Use standardization method given in *This Journal*, 24, 633.

(d) *Ferric sulfate solution*.—Dissolve 60 grams of  $\text{Fe}_2(\text{SO}_4)_3 + \text{Aq}$  in distilled water and dilute to 1 liter.

(e) *Ferric sulfate indicator*.—To 25% filtered solution of  $\text{Fe}_2(\text{SO}_4)_3 + \text{Aq}$  add approximately an equal volume of  $\text{HNO}_3$ .

#### PROCEDURE

Transfer 3 gram sample to 300 ml. Erlenmeyer flask. Add 50 ml. of 6% ferric sulfate solution (accurately measured with pipet or other accurately calibrated dispensing apparatus). Swirl flask during addition of the  $\text{Fe}_2(\text{SO}_4)_3$  to prevent caking of sample and to facilitate solution of chlorine. Add 100 ml. (also accurately measured) of 1+19  $\text{NH}_4\text{OH}$ . Swirl flask enough to insure solution of chlorine and thorough mixing of solution. (Very little swirling is necessary. If solution is agitated by vigorous vertical shaking, difficulty will be experienced in filtering.) Allow mixture to settle 10 minutes. Filter through dry No. 41 Whatman 11 cm. filter paper or through filter paper of approximately the same speed and retentiveness. Use 50 ml. aliquots (representing one-third of total) on samples low in chlorine (0–2% chlorine), and 25 ml. aliquots (representing one-sixth of total) on samples high in chlorine (over 2%).

If the approximate percentage of chlorine in the sample is not known, it may be well to take a 10 ml. aliquot for a trial titration. To this add 10 ml. of  $\text{HNO}_3$  + 10 ml. of the  $\text{Fe}_2(\text{SO}_4)_3$  indicator. Dilute to about 50 ml. Add 0.5 ml. of the KSCN solution and immediately add with stirring enough  $\text{AgNO}_3$  to entirely eliminate any reddish color. From this titration calculate the volume of  $\text{AgNO}_3$  necessary to precipitate all chlorine in the aliquot to be used, adding an excess equal to approximately 10% of total volume necessary although a somewhat greater excess will not affect results. A minimum total of 10 ml. should be used.

To the sample aliquot in a 250 ml. beaker, add 10 ml. of  $\text{HNO}_3$  and 10 ml. of the  $\text{Fe}_2(\text{SO}_4)_3$  indicator (or 20 ml. of a solution containing equal volumes of these solutions). Then add with stirring the calculated volume of  $\text{AgNO}_3$ . Heat to boiling and allow to cool to room temperature, stirring enough to coagulate precipitate. (Cooling may be hastened by immersion of beakers in cold water.) Titrate excess of  $\text{AgNO}_3$  with  $\text{KSCN}$ . End point is indicated by first appearance of reddish tint that persists for 15 seconds. For accurate work use a reference solution containing all ingredients except  $\text{KSCN}$ . End point is first change in color.

### COLLABORATORS

- L. V. Crowley, Massachusetts Agricultural Experiment Station.  
 E. J. Deszyck, Rhode Island Agricultural Experiment Station.  
 R. T. Merwin, Connecticut Agricultural Experiment Station.  
 R. Payfer, Department of Agriculture, Ottawa.  
 C. T. Smith, Massachusetts Agricultural Experiment Station.  
 C. H. White, Maine Agricultural Experiment Station.  
 R. L. Willis, New Jersey Agricultural Experiment Station.

In order to check the results shown in Table 1 with those obtained by using the proposed Grattan and Potvin method (*This Journal*, 23, 425), Payfer determined the chlorine in three samples by both methods.

Table 2 shows excellent agreement in results by the two methods and partially confirms the Associate Referee's observation, based on a limited number of analyses, that the method under present investigation measures the total chlorine in many plant materials used for feeding purposes.

TABLE 1.—*Collaborative results*

COLLABORATOR	CHLORINE (PER CENT)			
	DAIRY FEED	DAIRY FEED+ADDED KCl (A)	POULTRY FEED	POULTRY FEED+ADDED KCl (B)
Crowley	.78	3.93	.49	5.25
Deszyck	.75	3.89	.47	5.22
Merwin	.72	3.92	.49	5.20
Payfer	.75	3.87	.47	5.19
Smith	.82	3.95	.50	5.25
White	.77	3.94	.50	5.26
Willis	.75	3.89	.48	5.21
Average	.76	3.91	.49	5.23
Theoretical		3.88		5.19

TABLE 2.—*Chlorine in three samples by two methods*

METHOD	CHLORINE (PER CENT)		
	OTTAWA SAMPLE	COLLABORATIVE DAIRY FEED	COLLABORATIVE POULTRY FEED
Grattan and Potvin	2.86	.76	.46
Proposed A.O.A.C.	2.88	.75	.47

## COMMENTS OF COLLABORATORS

*E. J. Deszyck.*—The method is simple and yields check results. Combining the 10 ml. of  $\text{HNO}_3$  with the 10 ml. of  $\text{Fe}_2(\text{SO}_4)_3$  indicator would save one step.

*R. T. Merwin.*—The filtered solutions were clear and apparently free from protein; end points were quite definite and large excess of  $\text{AgNO}_3$ , even as much as 100%, does not affect accuracy of titrations.

*R. Payfer.*—(1) Personally we would prefer to avoid pipetting 100 ml. of ammoniacal solution. (2) Filtering is fast at the beginning but slow towards the end. (3) The amount of  $\text{AgNO}_3$  is quite large for the sample and this is not economical. (4) The method should state clearly what "low in chlorine" or "high in chlorine" means.

To obviate the pipetting of the ammonium hydroxide solution, Payfer suggests using a semi-automatic dispensing apparatus consisting of a 100 ml. pipet with the tip cut off and replaced by a three-way stop cock through which the pipet is filled and emptied. The stop cock is connected to a reservoir containing the ammonium hydroxide solution. A capillary tube is inserted in the top of the pipet through a small stopper to regulate the volume of solution. Laboratory supply houses offer several types of accurately calibrated semi-automatic equipment that would serve the purpose also.

The cost of the silver nitrate solution even when a large excess is used is negligible, being less than one cent per determination.

If the directions given in the procedure are followed carefully and the flasks recommended are used, filtration to obtain a clear aliquot will present no difficulty. Experience in this laboratory indicates that under proper conditions it should be possible to obtain enough filtered solution within five minutes to provide a suitable aliquot for the determination.

Since the accuracy of the method will not be impaired in any way, the suggestions offered by Deszyck and Payfer have been included in the method presented in this paper. This will eliminate the necessity for publishing these suggestions as changes in the method in later issues of *This Journal* as the Associate Referee will recommend that this method be adopted as official, first action, and that study of the method be discontinued.

The Associate Referee expresses his appreciation to the collaborators for their excellent work.

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REPORT ON MILK PROTEINS IN MILK CHOCOLATE

By MARIE L. OFFUTT (U. S. Food and Drug Administration  
New York, N. Y.), *Associate Referee*

The method proposed last year for the determination of milk protein in milk chocolate gave good collaborative results, and the method was adopted as official, first action.

This year two more samples of milk chocolate were sent out to collaborators for the determination of their milk protein by this method (*This Journal*, 25, 83). The chemists reporting and to whom acknowledgment is made are the following, connected with the U. S. Food and Drug Administration:

J. H. Bornmann, Chicago

H. Schuman, Philadelphia

Maryvee G. Yakowitz, San Francisco

J. H. Loughrey, Boston

W. O. Winkler, Washington

W. T. Mathis, Agricultural Exp. Sta.,

New Haven, Conn.

The results are shown in the table.

*Collaborative results on total milk protein*

COLLABORATOR	SAMPLE A CALCULATED 3.05		SAMPLE B CALCULATED 5.22	
	<i>per cent</i>	<i>Av.</i>	<i>per cent</i>	<i>Av.</i>
W. T. Mathis	3.47	3.47	5.00	5.01
	3.47		5.02	
W. O. Winkler	3.40	3.42	5.07	5.09
	3.44		5.11	
J. H. Loughrey	3.65	3.63	5.31	5.31
	3.60		5.30	
Maryvee G. Yakowitz	3.40	3.43	5.13	5.13
	3.45		5.13	
	3.45		5.13	
J. H. Bornmann	3.46	3.47	5.09	5.12
	3.47		5.14	
H. Schuman	3.23	3.28	4.89	4.95
	3.33		5.00	
Marie L. Offutt	3.34	3.33	5.22	5.23
	3.33		5.24	

The results this year again show good agreement among the analysts and also with the calculated amount of milk protein present.

## REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

The investigational problems relating to the quantitative separation and estimation of the oil-soluble food colors approved by the A.O.A.C. have not advanced to the collaborative stage. The Referee therefore pro-

posed a collaborative study of a rapid method for detection of tartrazine FD&C yellow No. 5 in alimentary pastes, because this problem has an added significance from the economic viewpoint at the present time. An additional consideration was the aptitude of detecting very small quantities of added dye in alimentary paste. With this objective, the Referee sent out to various collaborators sixteen sets of samples, each consisting of four subdivisions, and also a proposed method of procedure.

The samples were marked as follows:

- No. 1 Spaghetti—contained 2 p.p.m. of FD&C yellow No. 5
- No. 2 Spaghetti—no color added
- No. 3 Egg Noodles—contained 4 p.p.m. of FD&C yellow No. 5
- No. 4 Egg Noodles—no color added

The reports of the collaborators are as follows (tartrazine):

	Sample No.	
W. C. Woodfin Atlanta Station	1 — present	
	2 — absent	
	3 — present	
	4 — absent	
L. W. Ferris Buffalo Station	1 — present	} * positive } †
	2 — negative	
	3 — present	
	4 — negative	
J. J. Winston National Cereal Products Laboratory New York	1 — present	
	2 — no evidence	
	3 — present	
	4 — no evidence	
M. Tubis Philadelphia Station	1 — negative	
	2 — negative	
	3 — positive	
	4 — negative	
H. J. Fisher Connecticut Exp. Sta., New Haven, Conn.	1 — doubtful	
	2 — none	
	3 — trace of tartrazine	
	4 — none	
M. L. Offutt New York Station	1 — present	
	2 — none	
	3 — present	
	4 — none	
J. L. Hogan New York Station	1 — positive	} ‡ positive } †
	2 — negative	
	3 — positive	
	4 — negative	

\* Spot test on double dyeing.

† Coupling test.

‡ Spot test.

The following comments and criticisms were offered by the collaborators:

*W. C. Woodfin.*—1. Specify the strength of dilute alcohol used to wash magma.

2. Specify whether the dyed wool from the first dyeing should be combined.

3. Specify the strength of both alpha naphthol and alkali used for coupling.

*L. W. Ferris.*—Sample No. 3 appears to contain much more tartrazine than No. 1. There was no indication whatever of any tartrazine in Samples 2 and 4.

*J. J. Winston.*—In Sample 1 the test indicates a small amount of added color, the alpha naphthol coupling results in a light pink color. In Sample 3 obtained a good dyeing and coupling reaction.

*M. Tubis.*—In all cases it was difficult to obtain clean dyeing tests due to carbonization of the flour ingredients even when the original volume was maintained. The dyeing tests were all inconclusive. The recommendation is made that the alpha naphthol be fresh recrystallized. It was found that in most cases more than two 35 ml. portions of amyl alcohol were required. The coupling tests were weak when run simultaneously with tartrazine solution of about the same intensity.

*H. J. Fisher.*—First dyeings on wool showed a faint yellow dye from No. 3 and no color from any of the others. The dyeing came wholly from the washing of the amyl alcohol extract. Solutions appeared colorless in all cases and 10 ml. portions gave no color with alpha naphthol when treated according to directions. When the other 15 ml. were redyed on wool, No. 1 and No. 3 showed doubtful very faint yellow dyeings.

*M. L. Offutt.*—No difficulty was encountered in extracting the color, and the color reactions were definite when tartrazine was present.

*J. L. Hogan.*—The colors developed by the coupling test were in some cases not very strong, but still sufficiently conclusive to be sure of the presence or absence of tartrazine. However, I do not consider the spot tests very reliable; only by taking into consideration the intensity of the developed color on wool and the color of the unspotted wool was I able to reach the conclusions reported.

## DISCUSSION

The results indicate that the proposed method is applicable in detecting even very small quantities of tartrazine. The theoretical quantity of added dye present in 300 grams of Sample 1 was 0.6 mg., and in 300 grams of Sample 3 was 1.2 mg. Almost every collaborator was able to detect added color in Sample 1, some reporting considerable difficulty; however, not much trouble has been encountered in detecting tartrazine in Sample 3. Added importance must also be given to the fact that the magma mechanically absorbed a considerable amount of the dye with the result that the quantity of dye available was much less than the theoretical amount. An additional point of interest is the fact that although the collaborators were unfamiliar with the method, nevertheless satisfactory results were obtained. It may therefore be safely stated that with more experience, many of the recorded difficulties will be overcome.

The suggestions of the collaborators will be studied and if found to be practicable will be incorporated. It is the opinion of the Referee that a repetition of collaborative work on alimentary paste is advisable for 1943.

### *Qualitative Test for Yellow AB (FD&C yellow No. 3)*

The Referee offers the following simple tests for the identification of yellow AB (FD&C yellow No. 3). The color reaction appears to be



TABLE 1

COLOR 0.01 mg. (10 $\gamma$ )	CH <sub>2</sub> O 0.05 mL.	H <sub>2</sub> SO <sub>4</sub> 0.05 mL.	H <sub>2</sub> O 8.0 mL.	NH <sub>4</sub> OH SLIGHT EXCESS	CH <sub>3</sub> COOH SLIGHT EXCESS
Yellow AB	no change	pink	pink	light pink	pink
" OB	"	yellow	colorless	colorless	colorless
Oil Red XO	"	reddish orange	pink	orange red fluorescence	orange red
Oil Orange SS	"	"	"	"	"
Sudan I	"	yellow	orange yellow precipit.	orange yellow precipit.	orange yellow precipit.
Sudan II	"	orange	orange red precipit.	orange red precipit.	orange red precipit.
Sudan III	"	pink	pink	pink	pink, slight fluoresc.
Sudan IV	"	red	red	red	pink
Sudan Brown	"	light pink	light pink	light pink	light pink
Sudan G	"	orange yellow	orange yellow	orange yellow	orange yellow
Aniline Yellow	"	orange red	orange red	greenish yellow	orange yellow
Butter Yellow	"	scarlet	scarlet red	yellow	orange red
Oil Red SO	"	red	pink	pink	orange red

TABLE 2

COLOR 0.01 mg. (10 $\gamma$ )	CuPy 0.05 mL.	H <sub>2</sub> O 8.0 mL.	NH <sub>4</sub> OH 0.05 mL.	EXCESS ACETIC ACID
Yellow AB	yellow	pink	purple	colorless to yellow
Yellow OB	"	colorless	almost colorless	colorless
Oil Red XO	orange, slight fluoresc.	almost colorless to yellowish	bluish	orange red
Oil Orange SS	orange	almost colorless to yellow	bluish	orange red
Sudan I	yellow orange precipit.	yellow red, slight precipit.	yellow red, slight precipit.	yellowish red, slight precipit.
Sudan II	orange	reddish yellow	reddish yellow	reddish orange
Sudan III	pink	almost colorless	light bluish red	almost colorless
Sudan IV	pink	light pink	bluish	pink
Sudan Brown	orange	almost colorless	bluish red	light pink
Sudan G	orange yellow	yellow	yellow	yellow
Aniline Yellow	greenish yellow	greenish yellow	greenish yellow	greenish yellow
Butter Yellow	yellow	yellow	yellow	yellow orange
Oil Red SO	orange red	yellow green	bluish	bluish red

specific for this dye, and its sensitivity is 10 gamma ( $10\gamma$ ). The presence of yellow OB (FD&C yellow No. 4) does not materially change the color shade.

The procedure is as follows:

Test 1.—To 1 ml. of alcoholic solution of yellow AB containing 0.01 mg. of dye, add 0.05 ml. of U.S.P. formaldehyde solution (37%) and 0.05 ml. of strong  $H_2SO_4$ , mix, and add 8 ml. of water. A pink coloration, permanent for hours, will appear in about a minute. A stronger concentration of color produces a proportionally deeper shade. If strong ammonia is added to slight alkalinity, the color shade is not perceptibly altered and on further addition of excess acetic acid the original pink color is somewhat intensified. Yellow OB treated similarly is either colorless or slightly yellow.

The tabulated behavior of some of the ordinary oil-soluble dyes treated similarly is as follows:

Test 2.—Make a solution of 4.0 grams of copper sulfate  $\cdot 5H_2O$ , 10 ml. of pyridine, and water qs. 100 ml.

To 1 ml. of alcoholic solution of yellow AB containing 0.01 mg. of dye add 0.05 ml. of the above solution, mix, and add 8 ml. of water. A pink coloration, permanent for several hours, will appear in about a minute. If 0.05 ml. of strong ammonia is added, a purplish solution is obtained. Stronger concentration of color produces a proportionally deeper shade. Yellow OB treated similarly is either colorless or bluish.

The behavior of some of the ordinary oil soluble dyes treated similarly is shown in the tables.

#### *Rapid Method for Tartrazine in Alimentary Paste*

Place 300 grams of ground egg noodles or macaroni in a liter Florence flask with 250 ml. of 95% alcohol, 250 ml. of water, and 5 ml. of  $NH_4OH$ . Mix well and place on a covered steam bath (ca.  $70^\circ C.$ ) for 2 hours, shaking at intervals to prevent caking on the bottom of the flask. Remove, cool, and add 100 ml. of saturated salt solution. Centrifuge and decant the solution. To the magma add 100 ml. of dilute alcohol, mix well, and spin. Extract the combined solution twice with 100 ml. portions of gasoline to remove fats and oil-soluble colors. Place lower layer in casserole, gradually add 200 ml. of water, and acidify with 15 ml. of conc.  $HCl$ . Centrifuge off precipitate, and extract acid solution with two 35 ml. portions of amyl alcohol. Draw off aqueous solution in casserole, put in several small pieces of washed wool, and place on steam bath until a dyeing is obtained. Extract the amyl alcohol solvent with several small portions of water, transfer to a casserole, acidify slightly, add a few pieces of washed wool, and place on steam bath to dye. Remove wool from both casseroles. Wash the dyed cloth free of adhering foreign matter and transfer it to a smaller casserole with water containing a small quantity of ammonia and place on a steam bath until all coal-tar color is removed from the cloth.

Discard the wool. Evaporate the alkaline color solution to dryness on steam bath. Add 25 ml. of cold water to dissolve dye and filter. Divide the solution into ca. 15 and 10 ml. portions. Place the larger portion in a casserole and acidify slightly with conc.  $HCl$ . Put into the solution a small piece of washed wool to dye. Spot the dyed wool for tartrazine, comparing it with a standard of ca. identical intensity. Add to the smaller portion a drop of conc.  $HCl$  and excess bromine water. (A precipitate may form with bromine if a considerable amount of soluble protein matter is present. Centrifuge or filter off this precipitate to obtain a better reaction on coupling.) Destroy the excess bromine with aqueous hydrazine sulfate solution and couple immediately with alkaline solution of alpha naphthol. The formation of a pink or red coloring matter indicates the presence of tartrazine.

## REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS AND SYNTHETICS

By GEORGE L. KEENAN (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The substances chosen for study this year consisted of choline, sulfadiazine, and sodium sulfadiazine. The procedure followed was somewhat of a departure from that pursued heretofore, in that all samples sent to collaborators were control samples. It was considered, however, that the tests were quite distinctive for the substances submitted if the directions were followed. An expression of opinion from the collaborators would be as impressive with respect to the controls as if unlabeled samples had also been tested.

The directions for making the microchemical reagents and tests on these substances are as follows:

*Choline*

(Identified with Reinecke salt reagent)

*Reagent.*—*Reinecke salt.*—Dissolve 0.1 gram of ammonium reineckate and 0.03 gram of hydroxylamine hydrochloride in 10 ml. of 95% alcohol. Filter into a dropping bottle. Keep in refrigerator. The reagent is stable for at least 6 months.

*Preparation of sample.*—Prepare solutions of choline in distilled water in proportions of 1:100, 1:1000, and 1:10,000.

*Identification.*—Add a drop of acetone to a drop of a dilution of choline on a microscopical slide and stir with a clean glass rod. Then add a drop of the reinecke salt reagent and stir with a glass rod. Apply cover-glass and examine microscopically at a magnification of 100–150. In the case of the 1:100 dilution, thin, hexagonal plates and star-shaped forms will be produced in abundance. In the 1:1000 and 1:10,000 dilutions, the plates are six-sided but more coffin-shaped and not as numerous as in the 1:100 concentration. Rosette aggregates of these plates, frequently clustered with the plates arranged on edge and giving the appearance of needles, will be observed in some of the dilutions. The crystalline precipitate is choline reineckate.

*Choline*

(Identified with platinic chloride and sodium iodide)

*Reagents.*—*Platinic chloride*—5% in water; sodium iodide, 5% in water.

*Preparation of sample.*—Prepare a 1:100 and a 1:1000 solution of choline in distilled water.

*Identification.*—To a drop of 1:100 water solution of choline on a microscopical slide, add a drop of platinic chloride solution and stir preparation with a clean glass rod. Draw into one side of this drop a very small amount of the sodium iodide reagent. Add cover-glass and examine the preparation microscopically at a magnification of  $\times 200$ . At the dilution of 1:100 small, black, rectangular prisms will be formed in abundance, also slender black rods. At the dilution of 1:1000, however, the test does not appear to be sensitive, only an amorphous, black precipitate being formed (Fig. 2).

This test has already been described by Amelink,\* who mentions the

\* Amelink, F., "Schema sur mikrochemischen Identifikation von Alkaloiden," pp 43–45. Amsterdam (1934).

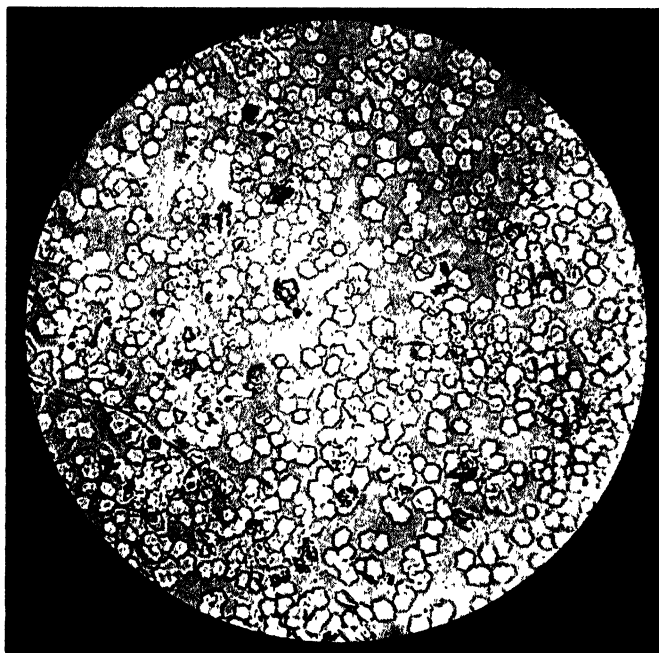


FIG. 1 - CHOLINE WITH REINECKE SALT REAGENT ( $\times 200$ ).

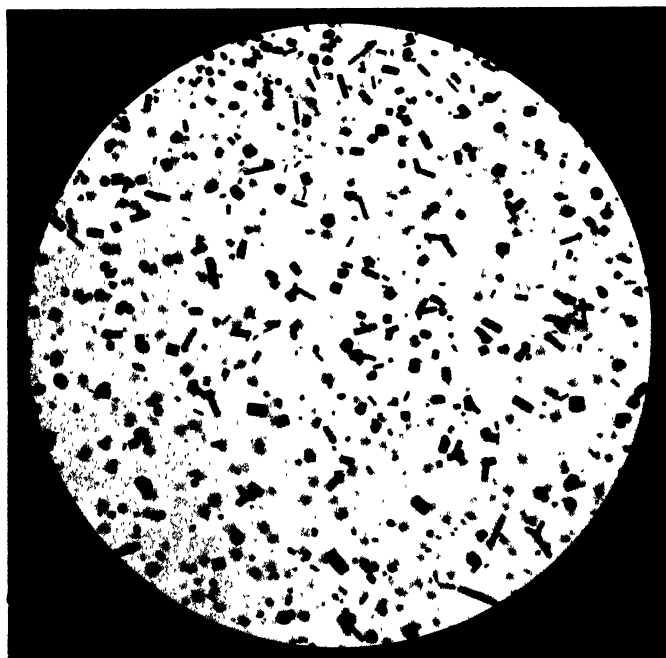


FIG. 2---CHOLINE WITH PLATINIC CHLORIDE AND SODIUM IODIDE ( $\times 150$ ).



formation of orange-brown plates and prisms with the addition of the platinic chloride reagent, but only upon evaporation of the preparation. The test with the two reagents, at a dilution of 1:100 of choline, was found to be much more satisfactory and distinctive.

#### *Sulfadiazine*

*Reagent.*—Gold bromide in HCl.—To 1 gram of gold chloride and 1.5 ml. of 40% HBr, add HCl to make 20 ml. (A saturated solution of NaBr may be substituted for the HBr.)

*Preparation of sample.*—Add a small amount of the substance (ca. 0.5 mg.) to 1 drop of distilled water on a microscopical slide, and stir into the drop. (Sulfadiazine is not very soluble in water but the latter serves as a satisfactory menstruum for purpose of the test.)

*Identification.*—Add a drop of the reagent to side of test drop, allowing it to flow in. Gradually there will appear floating on the solution, red, circular masses, which even at low magnification (about  $\times 15$ ) can be seen to consist of groups of very fine needles. As the slide stands, these groups become more numerous. On applying a cover-glass and examining the preparation at a magnification of  $\times 100$ , the aggregates will be found to consist of very fine needles with a brownish tinge. Mahogany-brown prisms will also occur in the preparation but the red aggregates of needles are most significant for the reaction. Sodium sulfadiazine, being more soluble in water, gives the test more quickly and furnishes more numerous aggregates.

### RESULTS AND COMMENTS

The following results and comments were made by collaborators:

*Chris K. Glycart, Food and Drug Administration, Chicago, Ill.*—The test for choline with reinecke salt reagent produced numerous six-sided plates. Crystals were also formed in dilutions to 1:10,000 as described. This is a remarkable test. The test with chlorplatonic acid and sodium iodide reagent was readily made; black rectangular prisms were observed best at the edge of the drop.

For sulfadiazine and sodium sulfadiazine, the numerous spherical clusters of mahogany-brown needles formed with reagent of gold bromide in hydrochloric acid is a distinctive test.

*Samuel Alfend, Food and Drug Administration, St. Louis, Mo.*—In general, the tests are considered good. There is no doubt that they are adequate for identifying choline and sulfadiazine. In the dilution 1:1000 for choline, small six-sided plates, and some coffin-shaped, were formed. In the 1:10,000 dilution, when the choline was added to the acetone, the reinecke reagent added, and the preparation stirred lightly many times, only hexagonal plates resulted. With the platinic chloride and sodium iodide test, small black rectangular plates were formed in the 1:100 dilution, these best seen at  $\times 200$ . In the case of the 1:1000 dilution, an amorphous precipitate was formed. When this was stirred vigorously, small black rods were obtained, best seen at  $\times 400$ .

*J. H. Cannon, Food and Drug Administration, St. Louis, Mo.*—In the case of choline, the results were good and the tests are apparently quite unaffected by slight variations in manipulations. In the case of sulfadiazine, the reagent recommended gave crystal masses striking in both size and color.

*Sam D. Fine, Food and Drug Administration, St. Louis, Mo.*—In the dilution of 1:1000 for choline, coffin-shaped forms were observed, and many six-sided thin plates. The acetone made it difficult to collect the crystals under the cover-glass. Crystals were best observed at the edge of the liquid, without cover-glass. With the

platinic chloride and sodium iodide reagent, slender, black rods were the most characteristic crystals observed. The 1:1000 gave an amorphous black precipitate.

For sulfadiazine, the gold bromide in HCl reagent showed aggregates of small needles floating on the surface of the test liquid. Similar needles were obtained with sodium sulfadiazine.

*F. M. Garfield, Food and Drug Administration, St. Louis, Mo.*—In the case of choline with the reinecke salt reagent, the characteristic crystals as described were obtained in both the 1:100 and 1:1000 dilutions. Very few crystals were found in the 1:10,000 dilution. With platinic chloride and sodium iodide, the crystals as described were obtained with the 1:100 dilution, but in the 1:1000 dilution, the precipitate appeared amorphous at  $\times 100$ , but was found to consist of definitely elongated rectangular prisms at  $\times 400$ .

*Jonas Carol, Food and Drug Administration, Chicago, Ill.*—The crystals in each case were easily identified. In the case of choline, the reinecke salt reagent was preferred.

*Irwin S. Shupe, Food and Drug Administration, Baltimore, Md.*—The tests appear to be satisfactory for the identification of these compounds. Choline with reinecke salt and in the 1:100 dilution gave very thin plates, formed first when stirred. In the 1:1000 dilution more perfectly shaped hexagons appeared. In the 1:10,000 dilution, no crystals were formed until there was considerable concentration from evaporation. In the case of the platinic chloride and sodium iodide reagent for choline, crystals were formed at the advance portion of the sodium iodide solution. No crystals were formed at the 1:1000 dilution. Sulfadiazine formed the groups of brown needles as described.

*J. Lincoln Perlman, State Food Laboratory, New York.*—In the microchemical identification of choline with reinecke salt, abundant characteristic thin hexagonal plates and star-shaped forms were noted at  $\times 100$ . In the 1:1000 dilution, mostly six-sided and coffin shapes were observed. The 1:10,000 dilution showed no star-shaped forms or aggregates. Dilutions above 1:20,000 yielded no crystals by the outlined procedure. With platinic chloride and sodium iodide reagent, abundant black rectangular prisms, some slender and rod-like, were observed at  $\times 200$ . At higher dilutions the test seemed of little value.

The detection of sulfadiazine and its sodium salt with gold bromide in HCl was of easy application and yielded characteristic results at  $\times 20$  to  $\times 100$ .

*Charles C. Fullon, Alcohol Tax Unit, Internal Revenue Service, Chicago, Ill.*—Reinecke salt gives a characteristic and very sensitive test for choline, although the crystals obtained from the 1:10,000 solution were not well formed. It is usually difficult to see the crystals readily, as they are very transparent and almost the same color as the solution. With crossed nicols they are a first order gray. I found the best way to see them was with crossed nicols and the "red plate," when they become blue and orange.

The platinic chloride-sodium iodide reagent for choline was not considered very satisfactory. In this test better results were obtained by using platinic bromide, then adding the sodium iodide as directed.

The test for sulfadiazine was found to work satisfactorily. However, assuming that dry substances were available, preference was expressed for the direct addition of a suitable reagent.

## SUMMARY

The comments of the collaborators indicate that the reinecke salt reagent gave the most distinctive and sensitive test for choline. Amelink's test with platinic chloride and sodium iodide is also useful and characteristic but not so sensitive as the reinecke salt test. The test for sul-

fadiazine was also found to be characteristic according to the outlined procedure.

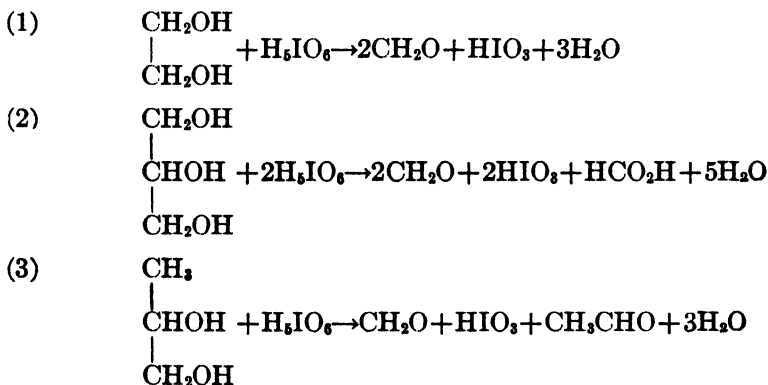
The Associate Referee desires to acknowledge the assistance of J. B. Wilson, Food Division, Food and Drug Administration, for suggestions in the preparation of the reinecke salt reagent.

## REPORT ON ETHYLENE GLYCOL, PROPYLENE GLYCOL, GLYCEROL, AND DIETHYLENE GLYCOL

By MILTON ORCHIN\* (U. S. Food and Drug Administration,  
Chicago, Ill.)

Malprade's classical investigation<sup>1</sup> on the oxidation of glycols with periodic acid provided chemists with a new and valuable reagent. Much work is now recorded in the literature on the use of periodic acid and its salts for the determination of the structure of organic molecules and for the quantitative determination of glycols. Recently Allen, Charbonnier, and Coleman<sup>2</sup> developed a method that specified periodic acid for the quantitative determination of mixtures of ethylene glycol, glycerol, and diethylene glycol, and in addition described a simple qualitative test for glycerol that the writer has used in the present work. More recently Hoepe and Treadwell<sup>3</sup> described a method for the quantitative determination of mixtures of glycerol, propylene glycol, and ethylene glycol, using potassium periodate.

The basis of the qualitative tests to distinguish between ethylene glycol, propylene glycol, glycerol, and diethylene glycol as outlined below, is the action of periodic acid on these glycols. Periodic acid oxidizes only those glycols that have two hydroxyl groups on adjacent carbon atoms; it will not react with a substance like diethylene glycol. The action of periodic acid on each of the substances is as follows:



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<sup>1</sup> *Bull. soc. chim.*, (4), 43, 683 (1928).

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 384 (1940).

<sup>3</sup> *Helv. Chim. Acta.*, 25, 35 (1942).



It will be noted that reactions (2) and (3) produce one different substance, while all three reactions produce formaldehyde. Thus if the oxidation of the glycol yields acetaldehyde, propylene glycol is present. If formic acid is produced, glycerol must be present. If neither formic acid nor acetaldehyde is formed but formaldehyde is present, ethylene glycol is indicated. If the glycol fails to produce formaldehyde on oxidation, diethylene glycol is indicated, and its presence can be confirmed by the preparation of a derivative. The derivative selected was the 3,5-dinitrobenzoate since it is easily prepared, and the 3,5-dinitrobenzoates have been suggested by the writer<sup>4</sup> as derivatives of other alcohols for A.O.A.C. purposes. Diethylene glycol can form a monobenzoate or a bis derivative. Actually the bis compound is easily formed, isolated, and purified, even when a large excess of diethylene glycol is present.

It is assumed that a few drops of pure glycol are at hand, and that this glycol is either ethylene glycol, propylene glycol, glycerol, or diethylene glycol. One drop of material (or its equivalent in aqueous solution) is a convenient quantity to use.

#### METHOD

*Reagents.*—(a) *Approximately 0.05 N periodic acid.*—Dissolve 11.0 grams of crystalline periodic acid in water and make to 1 liter.

(b) *Phloroglucinol reagent.*<sup>5</sup>—To 1 gram of phloroglucinol add 20 grams of NaOH, and water to make 100 ml.

(c) *Iodine solution.*—Approximately 0.1 N.

(d) *Crystalline 3,5-dinitrobenzoyl chloride.*

(e) *Dry pyridine.*

*Glycerol.*<sup>2</sup>—Add a drop of methyl red to ca. 5 ml. of the solution to be tested and neutralize with 0.1 N NaOH or 0.1 N HCl as required until the solution is just alkaline. Take ca. 5 ml. of periodic acid and adjust to the neutral point (of methyl red) with 0.1 N NaOH, leaving the solution just on the alkaline side. Mix the sample with the periodate solution and observe. If glycerol is present, the solution will turn red immediately. The reaction is due to the increased acidity arising from the formation of formic acid.

NOTE: This reaction can also be made the basis of a quantitative determination of glycerol.<sup>2</sup> The writer has analyzed over 50 samples containing glycerol, and the recoveries ranged from 98.5 to 100.5 per cent.

*Propylene glycol and ethylene glycol.*—If the test for glycerol is negative, add ca. 5 ml. of the periodic acid to ca. 5 ml. of the unknown in a test tube. After a minute, test for the presence of formaldehyde by adding 2 ml. of the phloroglucinol reagent. An immediate red color indicates formaldehyde. Either ethylene glycol or propylene glycol is responsible for the formaldehyde. To determine which of these is present, repeat the oxidation of the unknown. After 1 minute add an excess of 5% alkali followed by the iodine solution. If the unknown contains propylene glycol an immediate precipitate of iodoform will be present, arising from the acetaldehyde.

NOTE: Some samples of ethylene glycol give a faint odor of iodoform, probably due to minute traces of impurities, but no precipitate is obtained unless the sample is very impure.

<sup>4</sup> *This Journal*, 25, 839 (1942).

<sup>5</sup> *Methods of Analysis*, A.O.A.C., 1940, 462.

The presence of formaldehyde and the absence of acetaldehyde mean that ethylene glycol is present.

*Diethylene glycol.*\*—Reflux for ca. 15 minutes a solution of ca. 3 drops of pure diethylene glycol,\* 0.5 gram of 3,5-dinitrobenzoyl chloride, and 5 ml. of pyridine. While still hot, add a few drops of warm water. (On cooling the bis, 3,5-dinitrobenzoate of diethylene glycol separates.) After cooling thoroughly, filter, and wash successively with dilute HCl, water, ethyl alcohol, and ether. Recrystallize the precipitate from benzene-petroleum benzin, m.p. 150.5°–151.5°C. corr.†

## REPORT ON BARBITURIC ACID DERIVATIVES (Particularly Bromobarbiturates and Thiobarbiturates)

By L. E. WARREN (Food and Drug Administration, Federal  
Security Agency, Washington, D. C.), *Associate Referee*

Two years ago an associate referee was appointed to ascertain whether the official method for barbital and phenobarbital is applicable to other barbituric acid derivatives used in medicine. Considerable collaborative work was done, and as a result of the associate referee's report the official method, with slight modifications, was adopted as tentative for eight of the more commonly used barbiturates. These are: alurate, amytal, dial, evipal, ipral sodium, neonal, pentobarbital sodium, and phanodorn.

Last year the Association decided to continue the work and directed the associate referee to apply the modified method to certain other barbiturates not included in the first study, particularly the bromobarbiturates and thiobarbiturates, with the view to ascertaining whether the method already adopted is applicable. No collaborative work was to be conducted, except in doubtful cases.

Since the last meeting of the A.O.A.C. a revision of the Pharmacopoeia has been issued. It describes twelve barbituric acid derivatives or preparations containing them and prescribes assays for ten of them. In this study, however, no attempt has been made to include the Pharmacopoeial assays of the several barbiturates because of the great diversity of solvents directed by the Pharmacopoeia to be used. Some information concerning the U.S.P. assays is compiled for reference (Table 1).

Specimens of alphenal (Prophenal) powder and capsules, calcium ipral (powder and tablets), ortal sodium, pentothal sodium, pernoston, seconal sodium capsules, sandoptal, and thioethamyl sodium were obtained for investigation, and this year the adopted method was applied to market preparations of the barbiturates under consideration, to tablets and capsule contents, and to mixtures prepared to simulate tablets and capsule contents.

\* A pure sample of diethylene glycol was kindly supplied by the Union Carbide and Carbon Corporation.

† Huntress and Mulliken, "Identification of Pure Organic Compounds, Order I," John Wiley and Sons, p. 477, give the m.p. of bis-3,5-dinitrobenzoate of diethylene glycol as 149°C.

TABLE 1.—*Assay of several barbituric acid derivatives and preparations, according to U.S.P. XII*

SUBSTANCE	QUANTITY DIRECTED TO BE TAKEN	SOLVENT	STANDARD
	<i>gram</i>		<i>per cent</i>
Barbital	No assay	—	—
Barbital Tablets	Equivalent to 0.3	Alcohol 2 Ether 1 Chloroform 7	94–106 of labeled amt.
Barbital Sodium	0.5	Ether	88–90
Barbital Sodium Tablets	Equivalent to 0.3	Alcohol 2 Ether 1 Chloroform 7	94–106 of labeled amt.
Pentobarbital Sodium	0.5	Ether	90–92
Pentobarbital Sodium Tablets	0.3	Alcohol 2 Ether 1 Chloroform 7	90–105 of labeled amt.
Pentobarbital Sodium Capsules	Equivalent to 0.3	Alcohol 2 Ether 1 Chloroform 7	90–105 of labeled amt.
Phenobarbital	No assay	—	—
Elixir Phenobarbital	25 ml.	Chloroform	0.37–0.43 per 100 ml.
Phenobarbital Sodium	0.5	Ether	89–91.5
Phenobarbital Tablets	Equivalent to 0.3	Chloroform 80 Ether 20	94–106 of labeled amt.
Phenobarbital Sodium Tablets	0.13	Chloroform 80 Ether 20	90–105 of labeled amt.

## EXPERIMENTAL

As in the study last year, the acidic substances (if not obtained as such) were prepared from their sodium (or calcium) compounds by solution in water, acidification of the solution, agitation with chloroform, and evaporation of the solvent. The solubilities of the acidic substances in seven solvents were then studied. In general, all were soluble in alcohol, chloroform, and ether, and also in a mixture of chloroform and ether (4+1). All were relatively insoluble in petroleum benzin and water. Each was so slightly soluble in petroleum benzin that this solvent might be

employed with but slight error in extracting fatty lubricating materials from tablet mixtures before the active ingredient is extracted. The solubilities in benzene and carbon tetrachloride were variable, but in general they were less than in chloroform or ether.

There is a great diversity in the solubility of the barbituric acid derivatives in benzene. Some are relatively insoluble; others are quite soluble. Ipral, for example, requires over 2000 ml. for 1 gram. of substance, while ortal and neonal require only about 30 ml. and 40 ml., respectively. A few crystallize with benzene of crystallization. Pernostal is scantily soluble in chloroform (327 ml.) Some crystallize with difficulty (seconal) from any of the ordinary solvents.

The results for solubilities are given in Table 2.

The samples were subjected to two methods of analysis. Method I is the official method for barbital, phenobarbital, and several other barbituric acid derivatives, as modified by the Association at the 1941 meeting (*This Journal*, 25, 56).

Method I is as follows:

#### METHOD I

##### REAGENTS

(a) *Alkaline salt solution*.—Dissolve 20 grams of NaOH in water, dilute to 1 liter, add NaCl to saturation, and filter.

(b) *Solvent*.—Mix 20 ml. of ether and 80 ml. of  $\text{CHCl}_3$ .

##### DETERMINATION

Transfer 0.3 gram of powdered sample to a separator and dissolve in 10 ml. of the alkaline salt solution. If tablet lubricants (other than stearic acid) are present, wash with 15 ml. of ether and decant from top of separator. Repeat extraction with ether twice. Add 2 ml. of HCl to the alkaline solution, then 5 ml. of water to prevent precipitation of salt. Extract with  $\text{CHCl}_3$ -ether 5 times, using 30, 20, 20, 10, and 10 ml. portions of the solvent. Test for complete extraction by shaking with an additional 10 ml. of solvent and evaporating in separate beaker. Combine solvent in second separator and wash with 2 ml. of water acidified with a drop of HCl. Filter solvent through pledget of cotton into a small weighed beaker. Evaporate on steam bath with aid of electric fan, heat 10 minutes at 90°–100°C., cool in desiccator, and weigh. Reheat, cool, and reweigh until constant weight is obtained. Determine melting point to check purity of residue.

At the last meeting of the A.O.A.C. a vote was taken in the Drug Section to ascertain how many of the analysts present used pure chloroform in the assay of barbituric acid derivatives instead of the official mixture of chloroform and ether (4+1). The result indicated that nearly all used chloroform only; a few followed the Pharmacopoeial directions (U.S.P. XI) by using ether. Because of this expression of opinion, it was decided to employ chloroform only as solvent in one of the methods to be studied this year.

Method II is the same as Method I used last year (*This Journal*, 25, 803), except that chloroform alone is substituted as solvent for the mix-

TABLE 2.—*Solubilities of nine barbituric acid derivatives in seven solvents*  
(1 gram of substance soluble in stated number of ml. of solvent)

SUBSTANCE (ACIDIC)	ALCOHOL	BENZENE	CARBON TETRACHLORIDE	ETHER	CHLOROFORM	PETROLEUM BENZINE	WATER
Alphenal	Soluble	509.6 486.7	4,467 3,660	7.4 10.4	Soluble	17,452 17,679	580
Ipral	Soluble	2,407 2,207	17,600	57	11.7 12.0		590 570 577
Nostal	Soluble	1,400 1,165	949	31	800	40,379	1,307
Ortal	Soluble 1.40*	35.8 30.6	269 268	2.40*	1.45*	9,570	4,130* 3,028
Pentothal	Soluble	50.2	332 469	Soluble	Very Soluble	5,448	14,813 19,300
Pernoston	Soluble	217	653 730	Very Soluble	329.4 323.9	11,950 9,625	873 871 882
Sandoptal	Soluble	247.7 251.2	484.8	Very Soluble	Soluble	25,727	569.5
Seconal	Soluble	Soluble	Soluble	Soluble	Soluble	2,216	1,250*
Thioethamyl	Soluble	645 686	3,970 3,739 3,715	Soluble	Soluble	20,088	14,628 15,811

\* Data furnished by the manufacturer

ture of 80 volumes of chloroform and 20 volumes of ether. This method is as follows:

#### METHOD II

Weigh 0.3–0.5 gram of the sample into a separator, add 10 ml. of water and shake well. Add 5 ml. of 0.5 *N* NaOH and shake again. Acidify to litmus paper with 10% HCl, dropwise, and add ca. 1 ml. in excess. Shake out repeatedly with CHCl<sub>3</sub>, using 40, 30, 20, 20, and 10 ml. portions of the solvent. Test for complete extraction by shaking with an additional 10 ml. of solvent and evaporating in separate beaker.

Combine solvent in second separator and wash with 2 ml. of water acidified with a drop of HCl. Filter solvent through pledget of cotton into a small weighed beaker. Evaporate on steam bath with aid of electric fan, heat 10 minutes at 80°–90°C., cool in desiccator, and weigh. Add 2 or 3 ml. of anhydrous ether\* and evaporate the solvent. Dry at 80°–90°C., cool, and weigh. Repeat the treatment with anhydrous ether and evaporation until the weight becomes constant. Determine the melting point to check the purity of the residue.

Sodium was determined by evaporating the solution from which acidic substances (ortal, pentothal, seconal, thioethamyl) had been removed by Method II (without using 0.5 *N* NaOH) and heating the residue with an excess of sulfuric acid. The residue was treated with ammonium carbonate in the usual way before final weighing.

Since some of the market preparations are stated to contain an excess of sodium as carbonate, a determination of sodium may not be a reliable basis from which to calculate the proportion of sodium barbiturate present. Analysis shows also that some of the preparations contain less sodium than is required to form the monosodium compound. Some preparations contained less than the theoretical proportion of sodium.

Nitrogen was determined by the Kjeldahl-Gunning-Arnold method modified to use a reflux apparatus during the first stages.

Sulfur was determined by the Parr-bomb method.

Bromine was determined by the Parr-bomb method.

Each of the specimens and mixtures was subjected to analysis by each of the two methods previously described. The identity of the product was confirmed by the melting point and in some instances by determinations of bromine, sulfur, or nitrogen. The results are given in Table 3.

The melting points of the several substances as found in the literature and as determined in this study are recorded in Table 4.

#### COMMENTS

The results show that there is practically no choice between Method I and Method II. However, Method II is less likely to produce emulsions than is Method I. Method II has the advantage of a simpler technic. No sodium chloride is required, and no ether is used except for final drying. Because of the simpler technic and less likelihood of formation of emul-

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\* Usually two treatments with 2 ml. each of anhydrous ether were sufficient to remove the last traces of CHCl<sub>3</sub>, and to produce a crystalline residue. However, some of the acidic barbiturates are more difficult to secure in crystalline condition than others.

TABLE 3.—Analyses of several barbituric acid derivatives by two methods

SUBSTANCE	METHOD I		METHOD II		CALCULATED FROM BROMINE OR SULFUR		CALCULATED FROM SODIUM OR CALCIUM	
	FOUND	RECOVERY	FOUND	RECOVERY	FOUND	RECOVERY	FOUND	RECOVERY
Alphenal (Pro-phenal) capsules	67.64	100.2	68.53	102.6				
	67.69	100.3	68.60	102.7				
			68.04	101.8				
Alphenal mixture	69.27	100.7	69.24	100.2				
	69.15	100.1	69.24	100.2				
Calcium Ipral tablets	61.85	99.70	62.37	100.56				
	62.14	100.16	62.28	100.41				
Calcium Ipral	81.16	100.07	81.28	100.17			8.17	99.47
	81.04	99.92	81.02	98.85				
Nostal mixture* I	35.51	102.1	35.55	102.3				
	II 35.24	101.4	35.32	101.6				
Ortal Sodium	90.46	98.73	90.13	98.38			8.96	102.1
	89.64	97.56	90.20	98.46			8.92	101.7
Ortal mixture	57.72	100.7	58.04	101.2				
			57.29	99.83				
	58.61	102.2	57.40	100.1				
Pentothal Sodium	87.00	94.90	83.39	90.9	13.11	99.11	11.24*	135.1
	86.69	94.40	83.29	90.8	12.84	97.04		
			85.71	93.5				
Pentothal Sodium mixture	27.54	95.7	84.95	98.15				
	27.28	94.8	85.05	98.36				
Pernoston mixture	44.38	99.07	44.58	99.51				
	44.63	99.62	44.51	99.35				
Sandoptal mixture	24.88	101.6	25.18	102.8				
	24.61	100.5	24.66	100.7				
Thioethamyl Sodium	88.80	96.97	90.35	98.55				
	88.60	96.66	89.81	97.96			9.06†	104.1
			89.80	97.95			8.92	102.5
			88.80	97.95			9.08	104.2
Thioethamyl Sodium mixture	41.62	96.00	41.15	94.9				
	42.42	97.80	41.09	94.8				
Seconal Sodium mixture	38.44	105.2	37.32	102.8				
	36.81	101.3	37.84	104.0			3.67†	81.80
			36.33	100.0			3.43	76.38
			36.17	99.56			3.37	

\* Mixtures I and II were not identical.

† Some of the sodium compounds contained an excess of sodium in the form of sodium carbonate; others contained less than required to form the monosodium compound.

sions, Method II usually requires less time than does Method I. Owing to the scant solubility of pernoston in chloroform, Method I would seem to be preferable for the assay of pernoston preparations.

Under the conditions of the methods, three extractions with chloroform

TABLE 4.—*Melting points of several barbituric acid derivatives (acidic substance)*

SUBSTANCE	MELTING POINT (FOUND)	MELTING POINT (AS RECORDED IN THE LITERATURE)
	<sup>°</sup> C.	<sup>°</sup> C.
Alphenal	156–157.5	—
Nostal	176–177	177
Ortal	122–123	122
Pentothal	130–131.2	127–130
Pernoston	153–160	130–133
Sandoptal	137.5–138	136
Seconal	94–96	98–100
Thioethamyl	179–180	—

were usually sufficient to remove all the pentothal and thioethamyl from acidified solutions. Other barbiturates, like barbital, dial, nostal, and pernoston, which are less soluble in chloroform, required more shakeouts.

During the past several years, the Associate Referee has assayed specimens of most of the barbituric acid derivatives and their preparations as marketed. These examinations have included nearly all of the U.S.P. XII preparations. The results obtained show that all of these preparations may be assayed by using chloroform alone as solvent, or by the use of a solvent composed of 80 volumes of chloroform and 20 volumes of ether. The only barbiturates studied that might require a mixed solvent for extraction are nostal and pernoston. Nostal requires about 326 ml. of chloroform for solution of 1 gram of substance. Pernoston is very soluble in ether, and in a mixture of 80 volumes of chloroform and 20 volumes of ether it requires about 146.3 ml. for solution of 1 gram.

## REPORT ON HYDROGEN-ION CONCENTRATION OF FLOUR AND CEREAL PRODUCTS BY ELECTROMETRIC MEASUREMENTS

By F. A. COLLATZ (General Mills, Inc., Minneapolis, Minn.),  
*Associate Referee*

Bailey (*This Journal*, 11, 478) recommended the adoption of an electrometric method, utilizing the hydrogen electrode, for the measurement of hydrogen-ion concentration of wheat flours. This recommendation was approved and the method described by Bailey became official (first reading). The use of the hydrogen electrode, however, presented certain operating difficulties for the determination of *pH* of cereals, cereal baked products, and other biological material and was criticized because of the expense of equipment and time-consuming preparation for the test. Subsequently, the electrometric method was dropped as official and the colori-



metric method was adopted as official, first reading, in 1941 for the determination of H-ion concentration in cereal products, as recommended by Garnatz (*Ibid.*, 23, 482; 24, 65).

Further reports by Garnatz and his committee (*Ibid.*, 24, 583), in which the collaborators determined the pH of cereal products by both colorimetric and electrometric methods, indicated very close agreement of results by the two methods and also between the various individuals who participated in the study. It is significant that all but one of the collaborators who reported electrometric results employed some form of the glass electrode. The invention of this instrument is probably the most valuable development from a practical standpoint that has been made in the field of pH. It makes the measurement of pH in the extract of cereal products so simple, easy, and quick as to be comparable to the measurement of temperature with a thermometer. Furthermore, the cost of these pH electrometers is reasonable enough to remove the objection of high cost for this type of equipment. As a result of the collaborative work reported by Garnatz, he recommended that "further work be done to arrive at a satisfactory procedure for the determination of the hydrogen-ion concentration of cereal products by an electrometric method." The work reported by the present Associate Referee was directed toward the development of such a procedure.

Samples covering the range of pH usually encountered in cereal products were sent to the following collaborators:

- (1) W. F. Geddes, University Farm, University of Minnesota, St. Paul, Minn.
- (2) C. G. Harrel, Pillsbury Flour Mills, Minneapolis, Minn.
- (3) R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.
- (4) E. G. Bayfield, Kansas State College, Manhattan, Kan.
- (5) F. R. Schwain, Kroger Food Foundation, Cincinnati, Ohio
- (6) V. E. Munsey, Food and Drug Administration, Washington, D. C.
- (7) Pearl Brown, Perfection Biscuit Company, Fort Wayne, Ind.
- (8) F. A. Collatz, General Mills, Inc., Minneapolis, Minn.

#### DESCRIPTION OF SAMPLES

The following samples were submitted for collaborative study at two different times, and proximate analysis of the samples follows:

- (1) Short patent soft wheat flour
- (2) Spring wheat patent flour (unbleached)
- (3) Durum semolina
- (4) Bread (air dried and ground)
- (5) Biscuits (air dried and ground)
- (6) Crackers (ground)
- (7) Buffer solution: Phthalate buffer solution adjusted to pH 6.0 and sent as an unknown

A standard buffer solution, carefully adjusted to pH 4.0, was included with the above-mentioned samples to be used for standardizing the elec-

*Analysis of cereal samples (per cent)*

SAMPLE	ASH	PROTEIN N×5.7	MOISTURE	ETHER EXTRACT	CRUDE FIBER
1	.35	7.90	9.80	.87	.19
2	.42	13.00	13.40	.88	.22
3	.71	13.50	11.80	.72	.32
4	2.20	12.90	7.30	1.31	.68
5	3.52	9.10	8.80	10.30	.58
6	2.61	10.00	4.75	3.20	.51

trode and potentiometer system. This buffer was prepared by diluting 4.00 ml. of 0.1 *N* NaOH and 500 ml. of 0.1 *M* potassium biphthalate to 1 liter as directed by Kolthoff and Laitenen ("pH and Electro Titrations," p. 34, 2nd ed., 1941).

**METHOD**

Each collaborator was asked to determine the hydrogen-ion concentration, in terms of pH, of the six cereal samples submitted by each of the following methods:

A.—Weigh 10.0 grams of the sample into a clean, dry Erlenmeyer flask and add 100 ml. of recently boiled, distilled water at 25°C. Shake contents of flask until particles are evenly suspended and mixture is free of lumps. Digest for 30 minutes, shaking the suspension at frequent intervals. Let stand for 10 additional minutes, decant the supernatant liquid into the hydrogen-ion vessel, and immediately determine the hydrogen-ion concentration, expressing results in terms of pH and using an electrode and potentiometer that have previously been standardized by the standard buffer solution.

B.—Duplicate the procedure as given in A but filter the extract through folded, hardened filter paper, discarding the first 5 ml. and determining the hydrogen-ion concentration immediately as directed in A.

The results obtained by the collaborators with these two procedures are shown in Table 1. They indicate no significant difference in pH values obtained by the individual analyst by the decantation or filtration method. It is noticeable, however, that slightly closer checks are apparent on the filtered than on the decanted samples, considering the group as a whole. The simplicity of the decantation method and its advantages of speed outweigh this slight advantage, and filtering of the extract may be omitted.

The question of concentration or ratio of cereal to water for extraction was next investigated. Bailey (*This Journal*, 10, 469) indicated little variation in H-ion concentration when the ratio of flour to water was varied through a wide range and concluded that a ratio of 1 part of flour to 10 parts of water was suitable for this type of work. Halton and Fisher,<sup>1</sup> however, report an increase in H-ion concentration as the ratio of flour to water was increased. Accordingly a second set of samples was sent to

<sup>1</sup> *Cereal Chem.*, 5, 445 (1929).

TABLE 1.—*pH* results obtained by collaborators (A, cereal extract decanted, B, filtered)

COLLABORATOR	NO. 1		NO. 2		NO. 3		NO. 4		NO. 5		NO. 6		NO. 7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	4.88	4.93	5.99	5.85	6.38	6.40	5.35	5.42	7.29	7.26	8.34	7.60	6.03	6.03
2	4.99	4.97	5.90	5.92	6.30	6.24	5.52	5.55	7.34	7.32	8.04	8.04	6.00	6.00
3	5.07	4.92	6.05	5.80	6.43	6.20	5.62	5.50	7.40	7.22	8.45	7.70	6.12	6.12
4*	4.85	5.85	5.85	6.30	6.30	6.50	5.65	5.75	7.30	7.20	7.95	7.55	6.00	6.00
5	4.85	4.93	5.76	5.84	6.20	6.20	5.40	5.45	7.14	7.14	7.96	7.92	6.16	6.16
6	4.81	4.88	5.88	5.82	6.21	6.24	5.45	5.50	7.29	7.17	8.38	7.90	5.93	5.93
7	4.88	4.90	5.92	5.88	6.32	6.32	5.48	5.48	7.25	7.28	8.32	8.20	6.00	6.00
8	5.01	5.02	5.98	5.96	6.34	6.34	5.58	5.56	7.28	7.28	8.26	8.20	6.03	6.03
Av.	4.93	4.93	5.92	5.87	6.31	6.28	5.48	5.49	7.28	7.24	8.25	7.93	6.04	6.04
Max.	5.07	5.02	6.05	5.96	6.43	6.40	5.62	5.56	7.40	7.32	8.45	8.20	6.16	6.16
Min.	4.81	4.88	5.76	5.80	6.20	6.20	5.35	5.42	7.14	7.14	7.96	7.60	5.93	5.93
Diff.	.26	.14	.29	.16	.23	.20	.27	.14	.26	.18	.49	.60	.23	.23

\* Collaborator reported apparatus out of order, so results were omitted from averages.

TABLE 2.—*Effect of concentration on pH of cereal extracts where 10 grams of cereal product are extracted with A, 100 ml. of water and B, 50 ml. of water*

COLLABORATOR	NO. 1		NO. 2		NO. 3		NO. 4		NO. 5		NO. 6		NO. 7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	5.12	5.01	5.85	5.83	6.24	6.05	5.25	5.14	6.61*	6.40*	7.57*	7.25*	5.63*	
2	4.96	4.97	5.87	5.88	6.26	6.22	5.36	5.30	7.26	7.11	8.10	8.15	5.99	
3	4.94	4.99	5.92	5.92	6.30	6.28	5.40	5.39	7.30	7.25	8.00	8.24	6.04	
4	4.80	4.80	5.80	5.80	6.20	6.20	5.20	5.20	7.20	7.20	8.10	8.00	5.80	
5	4.91	4.90	5.99	5.94	6.36	6.23	5.41	5.30	7.33	7.19	8.35	8.31	6.03	
6	4.81	4.82	5.86	5.91	6.21	6.21	5.28	5.35	7.22	7.21	8.40	8.47	5.95	
7	4.81	4.82	5.81	5.88	6.28	6.25	5.22	5.26	7.20	7.17	8.30	8.28	6.08	
8	4.91	4.92	5.89	5.85	6.30	6.31	5.29	5.33	7.26	7.22	8.13	8.14	6.00	
Av.	4.90	4.90	5.87	5.87	6.27	6.22	5.30	5.28	7.25	7.20	8.19	8.22	5.98	
Max.	5.12	5.01	5.99	5.94	6.36	6.31	5.41	5.39	7.33	7.25	8.40	8.47	6.08	
Min.	4.80	4.80	5.80	5.80	6.20	6.05	5.22	5.14	7.20	7.11	8.00	8.00	5.80	
Diff.	.32	.21	.19	.14	.16	.26	.19	.25	.13	.14	.40	.47	.28	

\* Results omitted from averages.

Potentiometers used: Collaborator 1, Coleman 3C; 2, Coleman 3D; 3, Coleman 3D; 4, not reported; 5, Coleman 3D; 6, Beckman Type G; 7, Beckman Industrial; 8, Coleman, 3A.

the collaborators (duplicates of the first set) for study of concentration effects.

Method A, as described previously, was used as the standard procedure when the ratio of cereal product to water was 1:10 for extraction. Method B was a duplicate procedure of A, except that it called for 50 ml. of water to 10 grams of cereal product, a ratio of 1:5.

The data obtained on the second test (Table 2) indicate that over the range of concentrations tried, the results do not vary significantly in pH and show good agreement between the collaborators. Several collaborators indicated that a ratio of 1:5 was hard to work with, particularly on baked products, and preferred the higher dilution.

The data reported by Garnatz on the electrometric determination of pH in cereals, together with the data given in this report, establish the fact that the electrometric method is entirely reliable for this determination and gives excellent results in collaborative study. In the first test (Table 1), only one collaborator appeared to be seriously out of line in the results he submitted, and he questioned his own results as his apparatus was not working well. He did, however, report the results he obtained to avoid delaying the completion of the work. In the second set of samples (Table 2), his apparatus was working normally and his results are in good agreement with the average. In test number two one collaborator reported results of Samples 1, 2, 3, and 4 that were in good agreement with the other collaborators, but Samples 5, 6, and 7 were decidedly low and out of line with the average. Sample 7 was a buffer solution made up to pH 6.0, and it may be assumed that the apparatus was not in proper working condition for these samples.

It is recommended that procedure A, as given in this report, be adopted as a tentative method for the electrometric determination of H-ion in cereal products. It is further recommended that additional collaborative work be done on the decantation procedure versus the filtration procedure.

The Associate Referee wishes to acknowledge the splendid and prompt cooperation and help given him in this investigation by the collaborators listed. Acknowledgment is also made of the assistance of Raymond Koehn and Philip Sautier in the preparation and analysis of samples.

## REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory,  
New York, N. Y.), *Associate Referee*

In the work previously reported on this subject, *This Journal*, 25, 763 (1941), it was found that the Munson and Walker method is not suitable for the determination of reducing power because the solutions prepared

from low-grade molasses bumped violently during the reduction, causing boiling point variations, which in turn affect the amount of copper reduced. It was recommended that in the next series of analyses the Quisumbing and Thomas method, *Methods of Analysis, A.O.A.C.*, 1940, 138, in which the reduction is carried out at 80°C., be used and that the reduced copper be determined by the volumetric thiosulfate method or by the revised permanganate method of the Association, *Ibid.*, 501-502. New samples were sent out to the collaborators, one blackstrap (No. 1), one high-test molasses (No. 2), and one refinery molasses (No. 3), with the following directions:

(1) In the case of blackstrap molasses (No. 1) and refiners' sirup (No. 3), weigh out 12 grams, in the case of high-test molasses (No. 2), 8 grams. Transfer the sample to a 500 ml. wide-mouthed Erlenmeyer, using in all 75 ml. of water. Add 30 grams of coarsely chopped fresh Fleischmann's baker's yeast (free from starch, obtainable in packages of 1 pound), and mix thoroughly with the molasses solution. Close the flask with a stopper provided with a delivery tube, the other end of which is immersed 1 cm. below the surface of water in a beaker; or any other form of water trap may be used. Place the flask in a water bath or thermostat kept at 30°C., and allow to ferment for at least 4 hours, shaking the flask from time to time. When fermentation is complete, transfer the contents of the flask quantitatively to a 250 ml. volumetric flask (with molasses that do not foam badly the fermentation can be carried out directly in the 250 ml. volumetric flask). Clarify with 15 ml. of neutral lead acetate solution (20 grams of acetate in 100 ml. solution), make to the mark at 20°C., add 1 gram of dried filter-cel, shake well, and filter, discarding the first few milliliters of filtrate. Deleat the entire remaining filtrate with ca. 0.5 gram of finely powdered anhydrous potassium or sodium oxalate, add 1 gram of dried filter-cel, mix well, and filter again. Determine the copper reducing power in two 50 ml. portions of the final filtrate by the Quisumbing and Thomas method as given in *Methods of Analysis, A.O.A.C.*, 1940, on pages 138 and 139, Sections 44 and 45. However, the reduced copper is *not* to be determined according to Section 46 or 47 on page 139, but by the volumetric thiosulfate method, as described on page 501, Sections 40 and 41, or by the *revised* volumetric permanganate method, pages 501 to 502, Sections 42 and 43. Electrolytic reduction, pages 502 to 503, Section 44, may also be used, if preferred. Report the copper (Cu) found in each of these determinations.

Run a blank in exactly the same manner as described, but using water instead of molasses. Determine the copper-reducing power upon 50 ml. aliquots of the final filtrate, and report the copper found in each.

If the final filtrate is not sufficient for two 50 ml. aliquots, repeat the experiment and blank, using twice the amount of molasses, added water, yeast, and clarifying agents specified, and dilute after fermentation to 500 instead of 250 ml.

(2) The yeast and lead precipitate occupies such a large volume that it is necessary to correct the result for it. The Scheibler double dilution method is proposed for this purpose.

Run a second set of determinations and blank, exactly as described under (1), but at twice the dilution specified there. If in the first set you used 12, or 8, grams, respectively, of molasses, and diluted after fermentation to 250 ml., dilute the same quantity of molasses in the second set to 500 ml. If in the first set you used 24, or 16, grams, respectively, of molasses and diluted after fermentation to 500 ml., dilute in the second set to 1000 ml. Determine the copper-reducing power in two 50 ml. portions of the final filtrate by the Quisumbing and Thomas method, as

TABLE 1.—*Milligrams of unfermented reducing substances calculated as invert sugar, after fermentation with Fleischmann's baker's yeast*  
(Corrected for blank)

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.	SIMPLE DILN.	DOUBLE DILN.
I T. Ben Arnold, Baton Rouge, La.	61.0 60.4	27.1 25.9	22.3 23.3	9.5 11.4	88.9 89.6	42.1 42.5
II Carl Erb, New York, N.Y.	67.0 69.1	29.3 29.5	25.5 25.6 25.1	10.9 10.2 9.9	95.4 95.2	44.9 43.9 43.3
III F. M. Hildebrandt, Baltimore, Md.	76.4 74.3	33.2 34.6	27.1 26.5	10.5 11.1	93.8 94.9 92.2 95.4	42.3 42.3 41.3 41.3
IV D. J. Smith, Boston, Mass.	54.0 54.5	22.0 22.3	22.7 23.7	9.2 9.8	84.4 84.5	35.1 35.2
V Carl F. Snyder, Washington, D. C.	63.2 61.5 62.1 61.0	29.0 29.7 27.5 27.4	23.5 23.7 23.9 24.4 25.1 24.5	10.2 10.6 11.1 11.1	90.4 89.9 87.0 87.5	44.9 43.9 39.7 38.5 41.2 40.7
Averages						
I	60.7	26.5	22.8	10.5	89.3	42.3
II	68.1	29.4	25.4	10.3	95.3	44.0
III	75.4	33.9	26.8	10.8	94.1	41.8
IV	54.3	22.2	23.2	9.5	84.5	35.2
V	62.0	28.4	24.2	10.8	88.7	41.5
Grand Averages	64.1	28.1	24.5	10.4	90.4	41.0
Same, omitting IV	66.6	29.6	24.8	10.6	91.9	42.4

described above, and report the copper found in each of these determinations and in the corresponding blanks.

First check the Quisumbing and Thomas table (Table 17, page 685) by a few tests on pure dextrose. If necessary, adjust the temperature of the water bath so that correct results are obtained with the dextrose.

Five of the collaborators sent in reports, and the results obtained are shown in Table 1.

The anticipated improvement has been only partly realized. Although each individual collaborator checked his own results in most cases within fairly narrow limits, there are still wide discrepancies between the average values found by different workers for the two low purity products. In the case of the high-test molasses they were smaller, as would be expected.

TABLE 2.—Average results, corrected for volume of insoluble matter

ANALYST	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	INVERT SUGAR		INVERT SUGAR		INVERT SUGAR	
	mg.	per cent	mg.	per cent	mg.	per cent
I	47.0	1.96	19.5	1.22	80.4	3.35
II	51.7	2.15	17.3	1.08	81.7	3.40
III	61.6	2.57	18.1	1.13	75.2	3.13
IV	37.6	1.57	16.1	1.01	60.3	2.51
V	52.4	2.18	19.5	1.22	78.0	3.25
Grand Averages	50.0	2.09	18.1	1.13	75.0	3.13
Same, omitting IV	53.3	2.22	18.5	1.16	78.7	3.28

In the experiments with the Munson and Walker method reported previously, it was found useless to correct the results for the volume of the insoluble matter, derived partly from the molasses itself, but mostly from the yeast and the lead precipitates. But in the present series the corrected values were calculated by the formula  $R_1 \times R_2 / (R_1 - R_2)$ , where  $R_1$  and  $R_2$  are the figures obtained by simple and double dilution, respectively. The results are given in Table 2, in milligrams and also in per cent of invert sugar.

Collaborator IV obtained the lowest results on all of the three samples, while the sequence of the results of the other four analysts is irregular. Since this indicates the possibility of a systematic error in the analyses of Collaborator IV, the grand averages have been calculated in two ways, including and omitting the results of this collaborator. The same has been done in Table 3, in which the averages of each analyst's results are com-

TABLE 3.—Comparison of individual averages with grand averages, mg. invert sugar

ANALYST	SAMPLE 1	SAMPLE 2	SAMPLE 3	AVERAGE ERROR	MEAN DEVIATION
A. For all 5 collaborators					
I	-3.0	+1.4	+5.4	±3.3	+1.3
II	+1.7	-0.8	+6.7	±3.1	+2.5
III	+11.6	0.0	+0.2	±3.9	+3.9
IV	-12.4	-2.0	-14.7	±9.7	-9.7
V	+2.4	+1.4	+3.0	±2.3	+2.3
B. Omitting Collaborator IV					
I	-6.3	+1.0	+1.7	±3.0	-1.2
II	-1.6	-1.2	+3.0	±1.9	+0.1
III	+8.3	-0.4	-3.5	±4.1	+1.5
V	-0.9	+1.0	-0.7	±0.9	-0.2



pared with the grand averages. The average errors, without regard to sign, have been calculated for each collaborator, and also the mean deviations from the averages.

When the results of Collaborator IV are included, both the average error and the mean deviation for this collaborator are over twice as large as the maximum shown for any of the others. If his results are omitted, the sum of the average errors is reduced from 22.3 to 9.9. Collaborators II and V show the smallest average errors and the smallest mean deviations from the averages. It appears, therefore, that it is possible to approach the probable values quite closely by the Quisumbing and Thomas method. If the values are expressed as percentages of invert sugar, and the results of Collaborator IV are again omitted, the maximum deviation of the results of any of the other chemists from the grand average is 0.35 for the blackstrap, 0.08 for the high-test molasses, and 0.15 for the refinery molasses; the average deviations are 0.18, 0.06, and 0.09, respectively. By a little more practical experience with the Quisumbing and Thomas method the agreement should be bettered materially.

There is still the possibility that the results may have been influenced by the yeast, which was secured in widely scattered places. In further work to be carried out on this project it would be advisable to study the copper reduction and the fermentation separately. This could readily be done by carrying out reduction experiments on samples of distillery slop, which can be obtained in spray-dried form. After this question has been settled satisfactorily, the work with the molasses may be resumed, in order to decide whether the same brand of yeast produced in different places has any noticeable effect on the results.

It is recommended that in further work on this subject the determination of copper reducing power be investigated separately, with samples of commercial dried distillery slop, and that the study of the effect of the yeast be deferred until that problem has been solved.

## REPORT ON HAIR PREPARATIONS

By IRWIN S. SHUPE (Cosmetic Division, U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

This third report on the subject of hair dyes consists primarily of an investigation of methods for the determination of 2,5-diaminotoluene. Two methods were subjected to a collaborative study.

The first is a volumetric method developed by Herd (*This Journal*, **22**, 159) for the determination of paradiamines. As the result of last year's work, this procedure was adopted (*Ibid.*, **25**, 113) as official, first action, for the determination of *p*-phenylenediamine. Collaborative work was done this year to verify the applicability of this method for the determination of 2,5-diaminotoluene.

The second procedure under study depends on the extraction of the diamine and conversion to its diacetyl derivative. This process was previously proposed (3) for the isolation of diamines from certain mixtures (*Ibid.*, 24, 871).

#### METHODS

##### *Procedure I*

#### REAGENTS

(a) *Sodium hypochlorite*.—5% U.S.P. solution of NaOCl.

(b) *Sodium arsenite*.—10%. Dissolve 10 grams of C.P. Na arsenite in 100 ml. of water; or dissolve 8.5 grams of  $As_2O_3$  and 1.5 grams of NaOH in 100 ml. of water, heating to obtain solution.

#### DETERMINATION

To a separatory funnel containing 5 ml. of 5% NaOCl and ca. 1 gram of  $NaHCO_3$ , add, by means of a pipet or a buret, an aliquot of a solution of the sample representing 0.01–0.08 gram of diamine. If insufficient NaOCl is indicated by the presence of a brown color while the solution is being added, repeat the operation, using more NaOCl or a smaller aliquot. Thoroughly mix the solution during the addition of the sample aliquot by gently swirling the separatory funnel. After the charge has been added, stopper the separatory funnel and shake for ca. 10 seconds. Add 10 ml. of Na arsenite solution, stopper the separatory funnel, and shake again. Extract the dichlorimide with two successive 25 ml. portions of  $CHCl_3$  and combine the extracts in a second separatory funnel. Wash the combined extracts with 10 ml. of water and filter through a pledget of cotton into an iodine flask. Make an additional extraction, wash with the water, and combine with the major portion. Add 50 ml. of water containing 1 gram of KI and 3 ml. of conc. HCl to the combined  $CHCl_3$  extracts, stopper the flask, and shake vigorously for 1 minute. Titrate the liberated iodine with 0.1 N  $Na_2S_2O_3$ . Stopper the flask and shake vigorously at intervals during the titration. (The iodine in the  $CHCl_3$  acts as an indicator.) Toward the end of the titration add starch solution for the final end point. Each ml. of 0.1 N  $Na_2S_2O_3$  = 0.002035 gram of 2,5-diaminotoluene.

##### *Procedure II*

#### REAGENTS

(a) *Sodium hydroxide*.—(1+1). Dissolve 50 grams of reagent-grade NaOH in 50 ml. of water

(b) *Sodium sulfite*.—C.P. powder.

(c) *Ether*.—Anhydrous ethyl ether or U.S.P. ether, washed with water to remove alcohol. Wash the ether with several ml. of (1+1) NaOH solution. Use this alkali-washed ether for all extractions.

(d) *Acetic anhydride*.—U.S.P. reagent.

#### DETERMINATION

Transfer to an extractor a measured portion of an aqueous solution of sample representing 0.05–0.3 gram of diamine. Add ca. 0.05 gram of  $Na_2SO_3$  and the NaOH, equivalent to half the volume of sample. (The resulting solution should contain a minimum of 20% NaOH.) Extract with ether by means of a suitable continuous extractor or separatory funnels. If separatory funnels are used, make 5 extractions with 20 ml. portions of ether. Test for complete extraction with an additional portion of solvent.

Filter the ether extracts directly through a pledget of cotton into a tared dish.

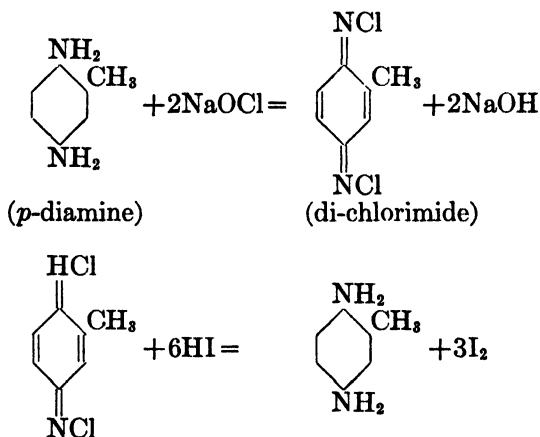
Add ca. 1 ml. of acetic anhydride and evaporate in a current of air. (Caution: Do not attempt to evaporate the ether on a steam bath, as this invariably causes decrepitation and mechanical losses.) After evaporation of the ether, add a few ml. of alcohol and evaporate on a steam bath until the odor of acetic anhydride is no longer perceptible. Dry at 100°C. for 30 minutes, cool, and weigh the diacetyl derivative of the diamine.

Diacetyl 2,5-diaminotoluene  $\times 0.592 = 2,5$ -diaminotoluene

#### DISCUSSION OF METHODS

*The Dichlorimide Titration.*—In procedure I, the paradiamine is converted to its dichlorimide with hypochlorite. The dichlorimide is extracted with chloroform after the reduction of excess chlorine with arsenite. The dichlorimide is then shaken with an acid aqueous solution of potassium iodide and liberates free iodine. The iodine is determined by titration with thiosulfate and corresponds to one molecule of diamine for six atoms of iodine.

The chemical changes involved appear to be represented by the following equations:



The titration of the iodine takes place in the presence of the regenerated diamine, which goes to the acid aqueous layer. If the aqueous layer is made alkaline, the diamine can be recovered by extraction with a suitable solvent.

As noted by Herd (*loc. cit.*), the method is theoretically applicable to all paradiamines. In addition to *p*-phenylenediamine and *p*-toluenediamine, the dichlorimide titration has been found suitable for 2,5-diaminoanisole.

Although meta- and orthodiamines do not titrate quantitatively, they do show significant interference, so that in mixtures some preliminary separation of paradiamines would be necessary.

The influence of sulfites in the method is of interest since liquid hair dyes commonly contain sufficient sulfite to delay preliminary oxidation

of the amines. It was found that sulfites cause low recoveries of diaminotoluene. The effects of varying amounts of sodium sulfite are shown in Table 1. (Sufficient excess of hypochlorite was used in these experiments to more than compensate for the reducing power of the sulfite added.)

TABLE 1.—*Effect of sulfite on dichlorimide titration*

EXP. NO.	2,5-DIAMINOTOLUENE PRESENT	Na <sub>2</sub> O <sub>2</sub> ADDED	COMPARATIVE RECOVERY
	mg.	mg.	per cent
1	27	None	100
2	27	10	97.5
3	27	50	94.5
4	27	100	91.2
5	27	100	98.5*

\* In experiment No. 5, the sulfite was removed by preliminary heating of the solution acidified with HCl.

Hair dyes have been found to contain sodium bisulfite equivalent to about one-half the weight of diamine present. On this basis the recovery of diamine would be about 97 per cent even if sulfites were not removed.

The order of addition of reagents in the chlorination of the diamine does not appear to make any significant difference in per cent recoveries. However, when the directions of the method were reversed and excess hypochlorite was added to the sample, a residual pink color was observed at the end of the titration. This pink color interferes somewhat with the starch end point. The addition of sample to the hypochlorite minimizes the formation of this color.

The results under A in Table 2 were obtained by directions in the method, and those under B by the addition of hypochlorite to the sample.

By the use of a chloroform solution of the diamine in place of a water solution, but otherwise the same titration procedure, recoveries of 95–97 per cent were obtained with 2,5-diaminotoluene.

A characteristic display of colors occurs when a chloroform solution of the paradiamine reacts with aqueous hypochlorite. At first a brilliant

TABLE 2.—*Effect of the order in which hypochlorite and sample are combined*

EXP. NO.	2,5-DIAMINOTOLUENE SULFATE PRESENT	RECOVERY 2,5-DIAMINOTOLUENE	
		A	B
	mg.	per cent	per cent
1	200	55.0	55.1
2	100	55.1	55.2
3	50	54.9	55.0
4	20	54.3	54.3
5	10	54.3	54.3

green-colored compound is formed in the chloroform layer. This color quickly turns violet and then to pale yellow on further mixing.

**Extraction Procedure.**—In samples containing mixtures, a preliminary isolation of diamines is often necessary. The proposed extraction procedure eliminates substances such as sulfites and aminophenols, which might otherwise interfere in subsequent tests. One of the important conditions of the method is the maintenance of a high concentration of alkali. This serves three major purposes. It depresses the solubility of the diamines in the aqueous phase and favors their ready extraction with the ether. It retains various aminophenols in the aqueous solution. It also prevents solution of the aqueous phase in the ether.

Experiments have shown that ether extracts from aqueous solutions containing 20 per cent or more of sodium hydroxide do not carry over any fixed alkali. However, the ether must be carefully filtered through cotton to remove any suspended alkali particles. If the alkalinity falls too low, considerable quantities of alkali are dissolved by the ether and are not removed by filtration.

It is not only unnecessary to wash the ether extracts with water, but it is disastrous for quantitative recoveries, because of the solubility of the diamines in the water layer.

After the diamine is separated by extraction it may be determined by several procedures. It can be transferred to an acid aqueous solution and then estimated with the dichlorimide titration. The free base may be weighed as such after evaporation of the solvent, although special care is required to prevent oxidation and absorption of moisture. For testing the efficiency of the extraction procedure, the simplest method appears to be a determination of the weight of the stable diacetyl derivative.

The diacetyl derivative of 2,5-diaminotoluene is too soluble to be quantitatively precipitated from chloroform as can be done with *p*-phenylenediamine (*loc. cit.*). An evaporation of the chloroform solution to dryness, however, will permit a quantitative recovery of the chloroform-soluble acetyl derivatives (*loc. cit.*).

#### COLLABORATIVE WORK

A specimen of 2,5-diaminotoluene sulfate and directions for determinations by procedures I and II were sent to collaborators.

TABLE 3.—*Analysis of collaborative sample of 2,5-diaminotoluene sulfate*  
[(C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>] Mol wt. 220.2

DETERMINATION	THEORY	FOUND
	<i>per cent</i>	<i>per cent</i>
H <sub>2</sub> SO <sub>4</sub>	44.5	44.6
Nitrogen	12.7	12.5
2,5-diaminotoluene	55.5	54.9

TABLE 4.—*Collaborative results on sample of 2,5-diaminotoluene sulfate*

COLLABORATOR	2,5-DIAMINOTOLUENE FOUND BY PROCEDURE—	
	I	II
1	<i>per cent</i>	<i>per cent</i>
	53.74	53.84
	53.70	54.10
	53.72	56.37
	53.65	55.24
		60.13
		100.31
2	54.09	56.27
	54.03	56.45
	54.14	
	54.05	
3	53.40	53.55
	(av. of 3)	(av. of 2)
4	52.95	53.93
	53.15	51.45
5	52.66	52.65
	52.70	55.06
6	54.1	54.9
	54.1	55.1
	54.2	55.0
7	54.25	59.26
	53.64	53.59
	54.69	53.97
	54.20	65.15
	53.58	64.31
	54.79	
8	54.51	
	53.48	54.49
	53.87	53.96
	53.92	
Variation	52.66–54.79	52.65–100.31
	Average 53.82	

The diamine salt sent out for analysis had been twice recrystallized from sulfuric acid and alcohol to remove most of the colored impurities. The dried recrystallized salt still had a light pink color and gave a light red solution in dilute acids. The diaminotoluene base isolated from the salt

had a melting point of 63°–64°C. as compared with the recorded temperature of 64°C. for 2,5-diaminotoluene. Comparisons of sulfate and nitrogen assays with the theoretical are shown in Table 3. The figure of 54.9 per cent for diaminotoluene base is the average of several determinations by procedures I and II.

The collaborative results of analysis are listed in Table 4 in the order in which they were received.

The Associate Referee is indebted to the following persons for their assistance and cooperation in testing the methods:

J. Bartholomew, Bourjois Mfg. Corp., Rochester, N. Y.  
Paul W. Jewel, Max Factor & Company, Hollywood, Calif.  
Louis Koch, H. Kohnstamm & Co., Inc., Brooklyn, N. Y.  
A. R. Norton, American Cyanamid Company, Bound Brook, N. J.  
F. H. Overton, Overton Laboratories, Hollywood, Calif.  
C. K. Glycart, Chicago, U. S. Food & Drug Adm.  
J. Carol, Chicago, U. S. Food & Drug Adm.  
G. M. Johnson, St. Louis, U. S. Food & Drug Adm.

The comments of the collaborators revealed difficulties with procedure II, but the extent of the difficulties is not fully represented in Table 4, since some of the figures were selected results.

Some of the more pertinent comments are as follows:

*Louis Koch.*—Procedure I is preferred because of its rapidity. The use of a continuous extraction apparatus (for II) gave findings that were so divergent that no mention of them has been made in this report.

*G. M. Johnson.*—Found extraction with separatory funnels entirely unsatisfactory and used a continuous extractor with better results. Noted carry-over of caustic alkali in ether extract.

*F. H. Overton.*—Found inconsistent results by procedure II.

*J. Bartholomew.*—Considered first method to be simpler and faster but found both methods to yield consistent results.

*Paul W. Jewel.*—Method II is not capable of giving consistently accurate results and procedure I is much to be preferred.

*A. R. Norton.*—The time required to perform procedure I is approximately 30 minutes and the time for procedure II is 2.5 hours.

## CONCLUSION

A collaborative study of the volumetric dichlorimide titration confirms the suitability of this method for the determination of 2,5-diaminotoluene in essentially pure form.

The second procedure studied was an extraction method useful for certain hair dye mixtures. The subsequent treatment with acetic anhydride yields a diacetyl derivative that is stable and has a melting point of value in the identification of the diamine.

However, the results and comments from the other chemists indicate that procedure II, as written, does not have sufficiently specific and detailed instructions. It is anticipated that a further study with more de-

tailed directions will show better results and permit the retention of the valuable features of the method.

## DETERMINATION OF 2,4-DIAMINOPHENOL

By IRWIN S. SHUPE (Cosmetic Division, U. S. Food and Drug Administration, Baltimore, Md.)

The most common, commercially available form of 2,4-diaminophenol is the dihydrochloride, known as "Amidol," and often used as a photographic developer. Its solutions are readily decomposed by oxidation and in alkaline solutions it reduces silver salts to metallic silver. Where it is mixed with magnesium carbonate and peroxide it dyes wool black. Both of these reactions are utilized in the dyeing of human hair.

In a study of general extraction procedures for hair dye amines,<sup>1</sup> diaminophenol was found to be one of the products not removed by immiscible solvents. Many other diamines and aminophenols may be differentiated and separated by their extractability.

The properties of certain salts of diaminophenol are of interest in consideration of possible quantitative methods. The hydrochloride is only slightly soluble in an alcoholic hydrochloric acid solution. The oxalate and phosphotungstate have low solubilities in water. However, these salts are unstable and cannot be dried with heat without decomposition.

Owing to the multiple functional groups in diaminophenol, the usual reagents for amines and alcohols are likely to yield several derivatives. In preliminary experiments with benzoyl chloride reagent, it was found that the weight of the derivative corresponded to dibenzoyl diaminophenol. Further investigation showed that a mixture of the tri- and dibenzoyl compounds was actually present. However, the constancy of the results obtained suggested its suitability as a quantitative procedure.

Since no suitable methods appear to be available for the determination of 2,4-diaminophenol, the procedures here described were developed. Certain microchemical tests are also included. These methods have been of value in confirming the identity of some related compounds that may be converted to diaminophenol.

### METHODS

*Benzoyl derivative.*—Transfer a quantity of sample equivalent to ca. 0.1 gram of 2,4-diaminophenol to a 250 ml. glass-stoppered Erlenmeyer flask. Add ca. 0.1 gram of  $\text{Na}_2\text{SO}_4$ , sufficient water to make a 25 ml. volume, ca. 1.5 grams of  $\text{MgCO}_3$ , and 1 ml. of benzoyl chloride. Shake vigorously. Open the stopper carefully to permit gases to escape. Shake at frequent intervals during 15 minutes, dilute with water to 100 ml., and add 5 ml. of conc.  $\text{HCl}$ . Bring to boiling over a burner and let stand on a steam bath for ca. 45 minutes. Filter the hot solution through a tared Gooch crucible, transfer, and wash the precipitate with ca. 150 ml. of hot water; dry at  $110^\circ\text{C}$ . for 1 hour and weigh.

<sup>1</sup> *This Journal*, 24, 872 (1941).



Wt. of derivative  $\times 0.374$  = diaminophenol.

Wt. of derivative  $\times 0.593$  = diaminophenol-dihydrochloride.

Mix a portion of the derivative obtained as directed above with ca. 10 or 15 ml. of 5% NaOH solution. Filter, and acidify the filtrate with HCl to precipitate the dibenzoyl derivative. Collect the precipitate on a filter and recrystallize from hot alcohol. Dry at 110°C. and determine the melting point.

N-N'-dibenzoyl-2,4-diaminophenol melts at 253°-254°C.

*Microchemical tests.*—To a drop of a 1% aqueous solution of 2,4-diaminophenol dihydrochloride, add a drop of one of the reagents listed in Table 1. Stir if no immediate precipitate forms. By means of a microscope (at ca. 100 $\times$  magnification) observe the characteristics of the crystals formed. Compare unknown samples with a control of 2,4-diaminophenol.

TABLE 1.—*Microchemical tests*

REAGENT	DESCRIPTION OF TEST
Ammonium oxalate (sat. aqueous solution)	Needles, singly and in groups after stirring
Picric acid (sat. aqueous solution)	Groups of long, curved blades after stirring
Kraut's reagent*	Small orange plates and burr-shaped crystals
Silicotungstic acid (10% aqueous solution)	Diamond-shaped crystals after stirring
Phosphotungstic acid (10% aqueous solution)	Very small rectangular prisms (best observed at 1-4000 dilution)

\* Dissolve 7 grams of bismuth subcarbonate in 20 ml. of conc. HCl. Add this solution to 28 grams of KI dissolved in 50 ml. of water, and dilute to 100 ml.

## DISCUSSION

The analytical method here described is particularly suited to hair dye mixtures containing magnesium carbonate and diaminophenol dihydrochloride. The experiments recorded in Table 2 were made with such mixtures.

TABLE 2.—*Recoveries of 2,4-diaminophenol calculated from weight of benzoyl derivative mixture*

EXP. NO.	2,4-DIAMINOPHENOL DIHYDROCHLORIDE	WEIGHT OF DERIVATIVE	RECOVERY BASED ON FACTORS FOR DIBENZOYL DERIVATIVE
	<i>mg. present</i>	<i>mg.</i>	<i>per cent</i>
1	50	86.9	103.0
2	75	126.3	99.8
3	100	167.1	99.1
4	100	169.1	100.3
5	100	170.0	100.8
6	150	253.9	99.6
7	200	340.6	100.9
8	250	424.2	100.5
9	300	501.3	99.0

Nitrogen determinations and other tests showed that the material obtained by the proposed procedure corresponds to about 21 per cent of tri- and 79 per cent of dibenzoyl diaminophenol. This represents a ratio of 1 mol of the tri- to 5 mols of the dibenzoyl compound. The actual recovery of diaminophenol nitrogen in the total precipitate was only about 95 per cent. Some of the analyses made on these compounds are shown in Table 3.

The mixture of di- and tri-derivatives obtained in the method softens at about 235°C. and liquefies at about 240°C. The sym-tri-benzoyl derivative (m.p. about 240°C.) is insoluble in cold aqueous alkalis, but is readily saponified to the dibenzoyl with alcoholic potassium hydroxide. The N-N'-dibenzoyl-2,4-diaminophenol is soluble in strong alkalis but insoluble in sodium bicarbonate. When recrystallized as directed it melts at 253°-254°C. This agrees with the findings of Meldola and Hollely.<sup>2</sup>

TABLE 3.—*Analyses of diaminophenol and benzoyl derivatives*

COMPOUND	HCl		NITROGEN	
	THEORY	FOUND	THEORY	FOUND
2,4-Diaminophenol dihydrochloride	per cent 37.0	per cent 37.0	per cent 14.22	per cent 14.25
Benzoyl derivative mixture (m.p. 235°-40°C.)			8.00*	7.94
N-N'-dibenzoyl-2,4-diaminophenol (m.p. 253°-4°C.)			8.43	8.42

\* Note: Based on 21% tribenzoyl and 79% dibenzoyl derivative.

The methods described have also been of value in connection with the characterization of some other related compounds. The reduction of 2,4-dinitrophenol, 2-amino-4-nitrophenol, 2-nitro-4-aminophenol with tin and hydrochloric acid converts the nitro groups to amines. The action of hydriodic acid on 2,4-diaminoanisole also yields diaminophenol. The identification of 2,4-diaminophenol as the main product in the reactions of these materials will aid in the establishment of their identity.

### SUMMARY

Procedures have been described for the preparation of benzoyl derivatives and for microchemical tests useful in the identification of 2,4-diaminophenol.

An empirical procedure based on benzoyl derivatives was found especially suitable for the estimation of 2,4-diaminophenol in hair dye mixtures containing magnesium carbonate. Recovery data on such mixtures have been included.

<sup>2</sup> J. Chem. Soc., 101, 931 (1912).

### CORRECTIONS

In the insert, *This Journal*, 25, 830(a), the legends should be transposed.

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In the note published in *This Journal*, 25, 783, the following precautions should be added: If a qualitative test for reducing sugars is positive, the official method of the A.O.A.C. for vanillin should be used.

It is important to adhere to the specified periods of time allowed for the precipitation of the resins and the dinitrophenylhydrazones so as to minimize any inversion of the sucrose that may take place.—MANUEL TUBIS.

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In the report of the Committee on Recommendations of Referees for the 1942 meeting, *This Journal*, 25, 68, it was recommended that the colorimetric method for the determination of lactic acid in milk and milk products, *This Journal*, 20, 134, be adopted as tentative. This reference should read "*This Journal*, 25, 255."

This change calls for a similar correction in Item 4 on page 85 of Volume 25 in the report on "Changes in Methods of Analysis."

## CONTRIBUTED PAPERS

### SPECTROPHOTOMETRIC STUDY OF THE GREEN COLOR IN PEAS

By H. FISCHBACH and S. H. NEWBURGER (Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

It is well known that conventional canning practices change the color of the fresh green pea to an olive green. J. S. Blair and T. B. Ayres<sup>1</sup> have developed a canning procedure in which they state that 60 per cent of the natural green color of the peas, chlorophyll, is protected against this change by inhibiting the rate of conversion of chlorophyll to pheophytin. This process maintains the peas in a mildly alkaline condition throughout the entire canning procedure by carefully controlled treatment with very dilute solutions of sodium carbonate ("presoak"), calcium hydroxide ("blanch") and magnesium hydroxide ("brine").

In view of certain provisions of the Federal Food, Drug, and Cosmetic Act relative to artificial coloring, especially of a type that is inherently deceptive, it was deemed advisable to conduct independent investigations designed to throw light on the question of whether the process involved the addition of any green pigment to that naturally present in the peas when they were harvested. Accordingly this paper deals with the spectrophotometric relationships that exist among the green coloring materials of the fresh pea, the conventional canned pea, and the "Blair processed" pea.

The pigment matter, presumably chlorophyll or its decomposition products, was extracted from the peas, and the transmission curves of these substances in ether solution were obtained with a General Electric recording spectrophotometer having an 8 m $\mu$  slit.

Because of the difficulty of interpreting the data from these crude extracts, derivatives were prepared. It was hoped that the transmission curves of such derivatives might indicate changes not apparent from the curves obtained with the crude extracts. In all this work no effort was made to estimate the color quantitatively.

#### HISTORY OF PEAS USED FOR ANALYSIS

1. Fresh peas of a sweet variety were purchased in season on the open market.
2. Blair processed peas of a sweet variety were obtained from a commercial packer in Wisconsin. These were analyzed approximately three months after they were canned. These peas resembled cooked fresh peas in color. The average alcohol-insoluble solids content was 14.72%.
3. Blair processed peas of Alaska variety were obtained from a commercial packer in Wisconsin. These were analyzed approximately four months after they were canned. Whereas the above sweet variety (No. 2) of peas was uniform in color

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<sup>1</sup> *Ind. Eng. Chem.*, **35**, 85 (1943).

this pack of the Alaska variety contained a number of pale olive green peas together with some pale yellow peas. These peas exhibited an average alcohol-insoluble solids content of 20.80%.

4. Peas of the Alaska variety, canned in the Midwest by the conventional commercial method, were analyzed about four months after they were packed. Canned Sweet and Alaska variety peas from various other sections of the country were also used, and similar results were obtained.

Prior to analysis, all of the canned peas were maintained in cold storage at 40°F.

#### PROCEDURE

*Extraction of Pigment.*—Wash 150–200 grams of shelled fresh peas or drained canned peas with copious quantities of tap water, transfer to a 15 cm. Büchner funnel, and wash first with ca. 500 ml. of distilled water and then with ca. 300 ml. of double distilled water. (This extensive washing of the raw material eliminates soluble salts, especially traces of copper, zinc, or iron salts, which may combine with any pheophytin present in the extracted pigment.)

Following the last washing, draw air through the peas for 5 minutes. Transfer a 100 gram aliquot of the washed and air-dried material to a Waring blender, add 100 ml. (125 ml. for fresh peas) of 95% cooled ethanol (40°F.) containing 0.5 gram of  $\text{Na}_2\text{CO}_3$ , and add a sheet of filter paper to facilitate subsequent filtration. Stir the mixture at high speed for 5 minutes, filter through a 24 cm. fluted filter paper or a 4 inch Büchner funnel, and wash the residue with two 25 ml. portions of 95% ethanol. Transfer the filtrate to a 500 ml. Pyrex separatory funnel, add 50 ml. of ethyl ether, and shake the mixture vigorously. Introduce 125 ml. of a brine solution and shake the mixture carefully with a rotary motion. (Brine solution = 40 grams c.p.  $\text{NaCl}$  plus 5 grams c.p.  $\text{Na}_2\text{CO}_3$  made up to 1000 ml. with double distilled water.) Discard the aqueous phase and wash the ethereal phase with an additional 100 ml. of the brine solution. Analyze the crude ethereal phase in the spectrophotometer.

(The method of Blair and Ayres for extraction of the crude ethereal phase was also tried, and similar results were obtained. The above method was used for the sake of simplicity and reagent economies.)

*Cold Saponification Products.*—Treat the crude ethereal phase with 5 ml. of a saturated solution of  $\text{KOH}$  in methanol; shake vigorously, and allow to stand overnight in cold storage at 40°F. The next morning transfer the mixture with 20 ml. of double distilled water to a 125 ml. Pyrex separatory funnel. Discard the yellow ethereal phase, and wash the aqueous phase with an additional 25 ml. of ether. Dilute the pigment solutions to approximately similar color intensities and analyze in the spectrophotometer.

NOTES: Chilled ethanol was used to offset the heat imparted to the mixture by the motor of the Waring blender.

Double distilled water was prepared in Pyrex.

Any allomerism that occurs during the long contact of the pigment with ethanol will not interfere with the subsequent preparation of the potassium chlorophyllin salts.

Depending on the history of the pea, the aqueous phase consists of varying amounts of potassium chlorophyllin and derivatives of pheophytin.

The observed wave lengths were corrected to within  $\pm 2.0 \text{ m}\mu$  of their true values with the aid of didymium glasses tested by the National Bureau of Standards. A comparison of the major absorption peaks (Table 1) of the crude ethereal extracts shows that there are some small differences among them. The absorption peaks increase in wave length in the order of fresh, Sweet variety Blair, and conventional peas. However,

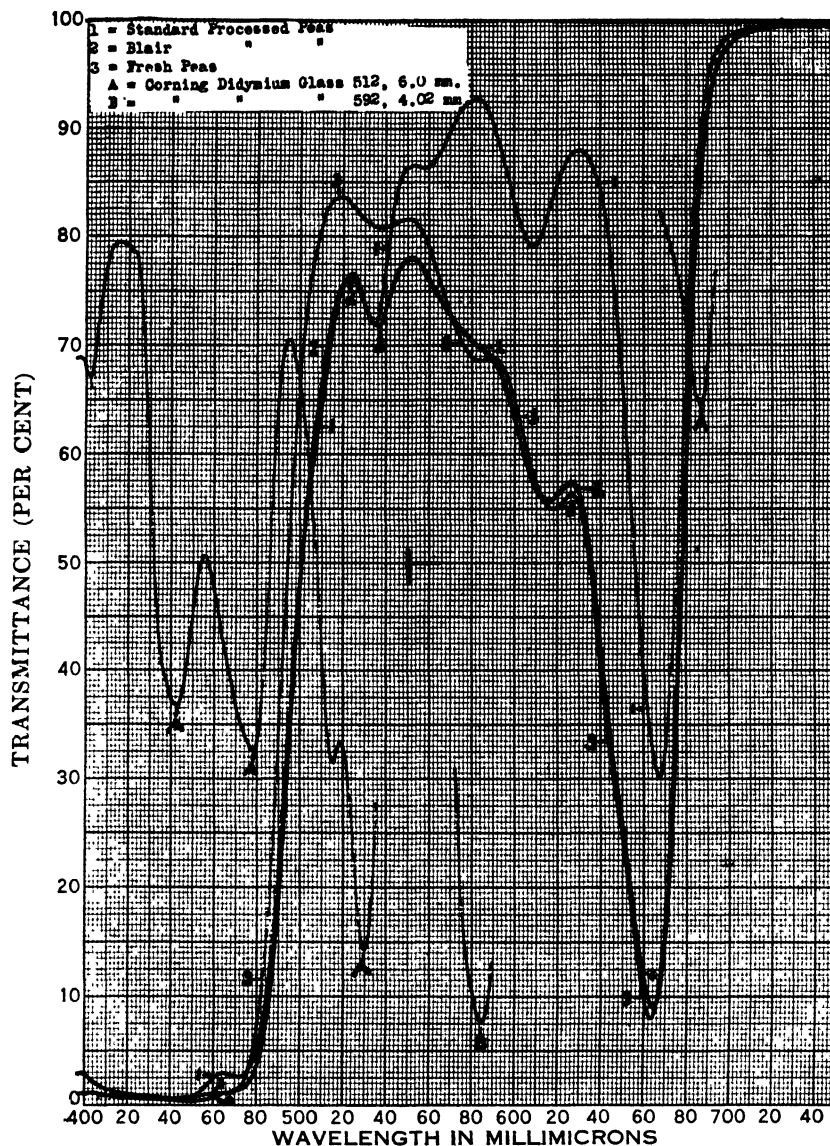


FIG. 1.—CRUDE ETHEREAL EXTRACTS.

TABLE 1.—*Crude ethereal extracts (Fig. 1)*

VARIETY	MAJOR ABSORPTION PEAK	MINOR ABSORPTION PEAKS
	m $\mu$	m $\mu$
Fresh Pea	661.4	616.5, 582.7, 536.0
Blair Processed Pea (Sweet Variety)	662.4	613.6, 535.0
Conventional Canned Pea	666.3	607.6, 534.0

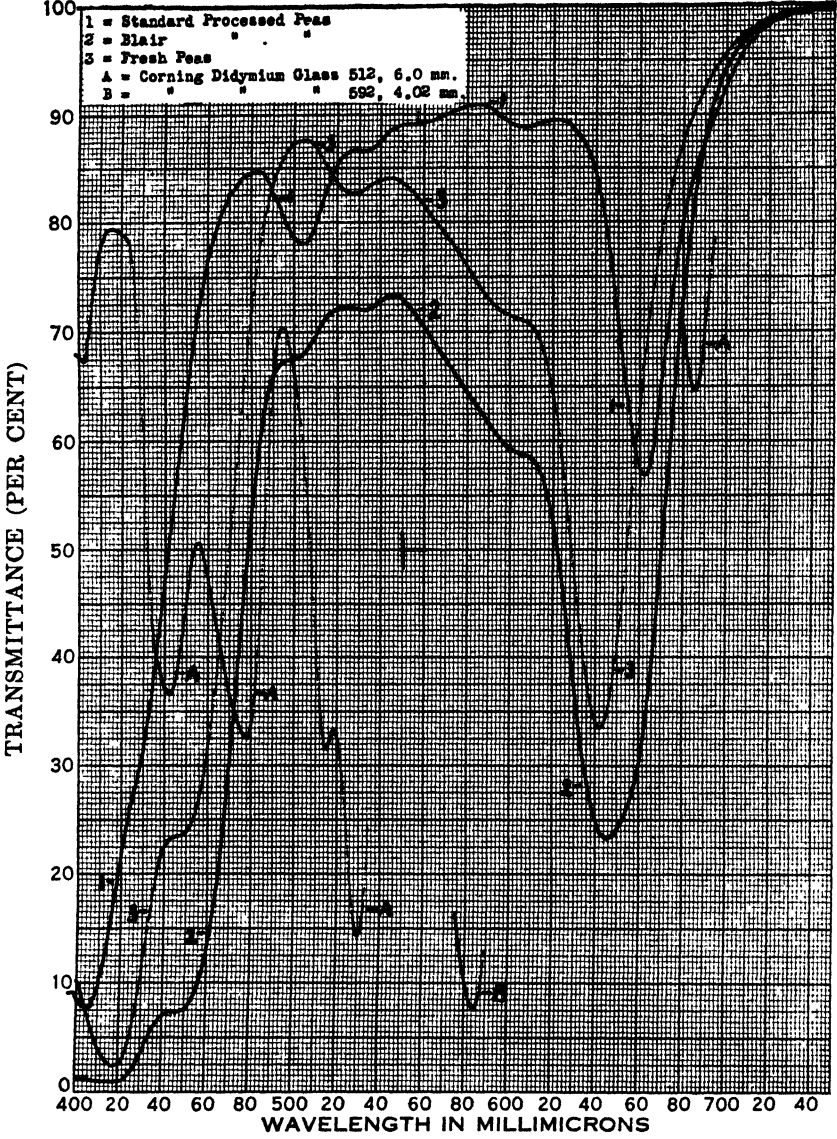


FIG. 2.—COLD SAPONIFICATION PRODUCTS.

TABLE 2.—Cold saponification products (Fig. 2)

VARIETY	MAJOR ABSORPTION PEAK	MINOR ABSORPTION PEAKS
	mμ	mμ
Fresh Pea	640.7	526.0
Blair Processed Pea (Sweet Variety)	643.7	532.0
Conventional Canned Pea	660.7	503.0





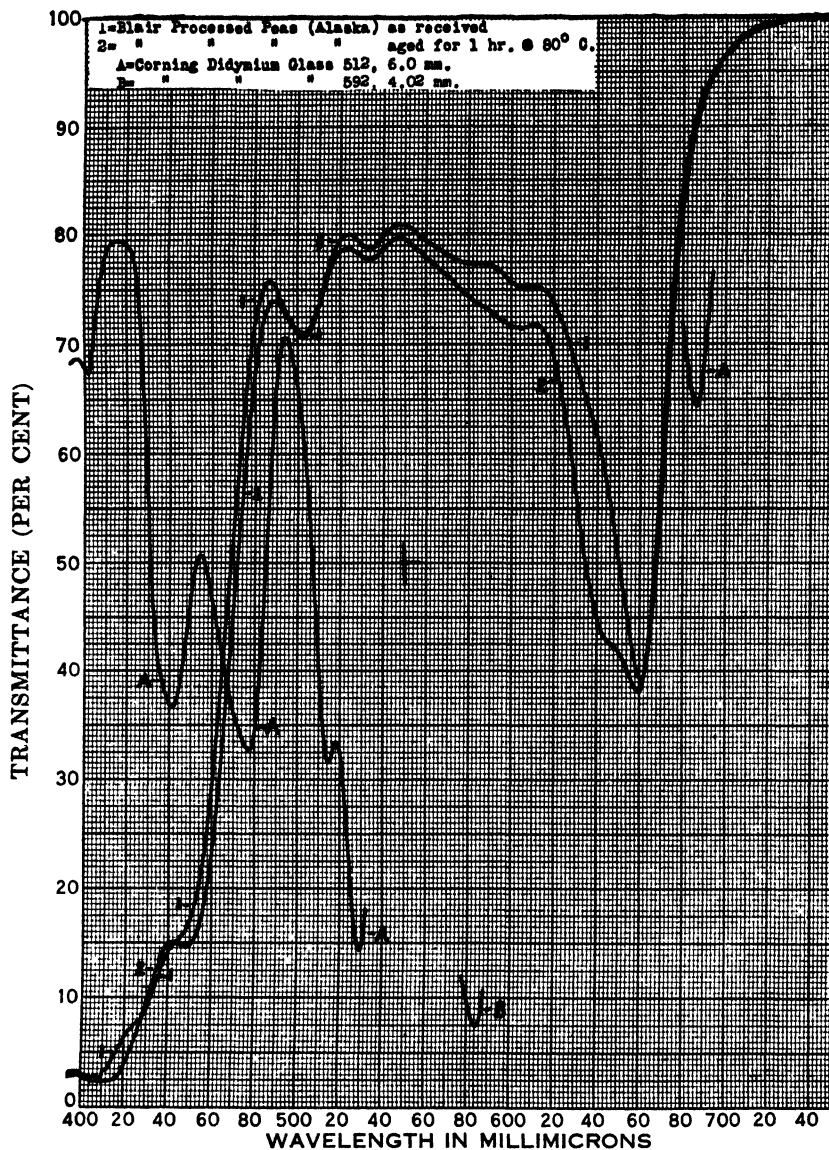


FIG. 4.—COLD SAPONIFICATION PRODUCTS.

the products of the cold saponification of the crude extracts exhibit significant shifts to shorter wave lengths for the fresh and Sweet variety Blair, but not for the conventional canned pea (Table 2). This shift can still be observed if the Sweet variety Blair pea is artificially aged by pre-heating the closed container for 1 hour at 80°C., but not after the pea is heated for 16 hours at 80°C. (Table 3). The Alaska variety Blair pea used

in this investigation gave no indication of absorption at a shorter wave length when its crude ethereal extract was saponified (Table 3).

#### DISCUSSION

Blair and Ayres have shown that as the organically combined magnesium of the green coloring material of canned peas diminishes, the color becomes more olive. They have also shown that the olive colored conventional packed pea contains no magnesium. This corresponds to the chemical transformation of chlorophyll to pheophytin. The following conclusions can therefore be drawn from the spectrophotometric data: If both the crude ethereal extract and the cold saponification products of the green coloring matter have absorption peaks in the region of 658.5–666.5  $m\mu$ , the canned pea has an olive color, and indications are that little if any organically combined magnesium was originally present. This of course precludes the presence of material amounts of chlorophyll. On the other hand, if the ethereal extract has an absorption peak in the region 661.5–662.5  $m\mu$  and the cold saponification products, one at 640.5–645.0  $m\mu$ , the presence of organically combined magnesium is indicated and the original color of the fresh pea has undergone little change. These conclusions were confirmed by preheating the Sweet variety Blair pea, a procedure that increases the rate of conversion of chlorophyll to pheophytin. The Alaska variety Blair pea has substantially less chlorophyll than have the Sweet varieties. The presence of the yellow peas observed in this particular pack denotes an even greater lack of chlorophyll than generally encountered in the average Alaska pea.

During the course of this work the phytochlorin and phytorhodin derivatives of the pea pigment from the different sources were analyzed but no significant variations were revealed by their transmission curves.

The question arises as to whether the major absorption peak of the cold saponification products occurs at only 640.5–645.0  $m\mu$  or 658.5–661.0  $m\mu$ . In the opinion of the writers this peak for the cold saponification products of the extracted green pigment lies in the region of 640.5–645.0  $m\mu$  when chlorophyll constitutes the major portion of the pigment, as exemplified in Figures 2, 3, and 4. It is further believed that as the proportion of chlorophyll in the pigment is reduced, the maximum absorption peak of the cold saponification products will shift progressively toward the region of 658.5–661.0  $m\mu$ . At some later date a pack of Sweet variety Blair pea of known history will be aged artificially and the relationship observed between the amount of magnesium (directly related to the chlorophyll content) and the position of the major absorption peak of the cold saponification products.

The spectrophotometric results of the artificially aged Blair peas substantiate the conclusion of Blair and Ayres;<sup>1</sup> to wit, that the new procedure for canning peas simply retards the rate of conversion of chlorophyll to

pheophytin (the rate at which the natural green pigment is converted to an olive drab color).

#### SUMMARY

Transmission curves in the spectral region of 400–750  $m\mu$  have been obtained for the crude ethereal extract and the cold saponification products of the green pigment in the fresh pea, the conventional canned pea, and the Blair processed pea.

An attempt has been made to correlate the spectrophotometric data with the color and organically combined magnesium content of the pea.

A plan for further work has been outlined.

#### ACKNOWLEDGMENT

The authors wish to express their appreciation to V. B. Bonney, of the U. S. Food and Drug Administration, for his interest and suggestions during the course of this study.

### SPECTROPHOTOMETRIC STUDY OF THE GREEN COLOR IN OKRA\*

By H. FISCHBACH and S. H. NEWBURGER (Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

Blair and Ayres<sup>1</sup> have shown that both heat and acid media change the normal green color of peas to an olive green. Canned okra, though subjected to conditions comparable to those employed in canning peas retains a green color. The writers undertook to investigate what role, if any, the standard practice of adding small quantities of zinc salts to the okra played in this phenomenon. In a previous paper (see p. 127) it was demonstrated that the natural green coloring matter of peas could be studied with the aid of spectrophotometric data. A similar procedure was followed with authentic packs of okra that had been prepared with and without the addition of zinc salts. The ethereal solutions and cold saponification products of the extracted pigments of these packs, as well as those of fresh okra, were analyzed in a General Electric recording spectrophotometer having an 8  $m\mu$  slit.

In the usual commercial method of canning, the raw okra is allowed to ferment overnight in a wooden vat containing a warm salt solution. It is then drained, trimmed, sorted, blanched, cut into pieces, filled into cans with a brine containing small quantities of zinc chloride, sealed, and processed.

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\* The work reported in this paper was performed because of its bearing on standards of identity for foods.

<sup>1</sup> *Ind. Eng. Chem.*, 35, 85 (1943).

## HISTORY OF OKRA USED FOR ANALYSIS

The packs of canned okra, identified as "A," "B," and "C," were prepared in the same plant.

*Sample A* was canned okra prepared by the above method. The color of the canned okra was "Apple Green" (Plate XVII)<sup>2</sup> and the pH was 4.8.

*Sample B* was prepared similarly to *Sample A* except that the packing medium was plain water. The color of the canned okra approximated "Grape Green" (Plate XLI)<sup>2</sup> with occasional pieces resembling "Apple Green" (Plate XVII).<sup>2</sup> The pH was 5.35.

*Sample C* was not subjected to any treatment in brine solution but was simply blanched, cut, packed in plain water, sealed, and processed. The color of the canned okra varied from "Dark Olive Buff" (Plate XL)<sup>2</sup> to "Citrine Drab" (Plate XL)<sup>2</sup> and had a pH of 5.75.

*Sample D* was fresh okra purchased on the Washington, D. C., market. Its color was "Mineral Green" (Plate XVIII).<sup>2</sup>

## PROCEDURE

The core and seed of the okra were removed, and the fleshy, green portion was placed in a Waring blender and treated as previously outlined in the paper on canned peas (*loc. cit.*).

NOTE: A 4% NaCl solution was used for washing the ethereal phase. Sodium carbonate was eliminated from the alcohol and salt solutions to prevent the precipitation of "tannates."

The observed wave lengths were corrected to  $\pm 2.0$  m $\mu$  of their true values with the aid of didymium glasses, tested by the National Bureau of Standards.

The major absorption peaks of the crude ethereal extracts all lie in the spectral region of 658.9–666.4 m $\mu$ . However, the products of the cold saponification of the crude extracts exhibit significant shifts to shorter wave lengths for the fresh and zinc processed okra (640.8 m $\mu$  and 640.3 m $\mu$ ), but not for the fermented and unfermented okra (661.3 m $\mu$  and 661.8 m $\mu$ ). This shift persists even when the zinc processed okra is heated in the closed container for 16 hours at 80°C.

## DISCUSSION

The major absorption peaks of the okra ethereal extracts show a striking similarity to those obtained with fresh and canned peas. By analogy with the peas, the spectrophotometric shift of the cold saponification products of the fresh okra can be associated with the presence of organically combined magnesium in the green coloring matter, chlorophyl. However, the heat treatment necessary for processing the canned okra, together with the acid packing medium present, should have effected the breakdown of the magnesium-bearing chlorophyl.<sup>1</sup> The fact that the zinc treated canned okra could be heated for 16 hours at 80°C. without altering the spectrophotometric characteristics of its pigment is additional proof that

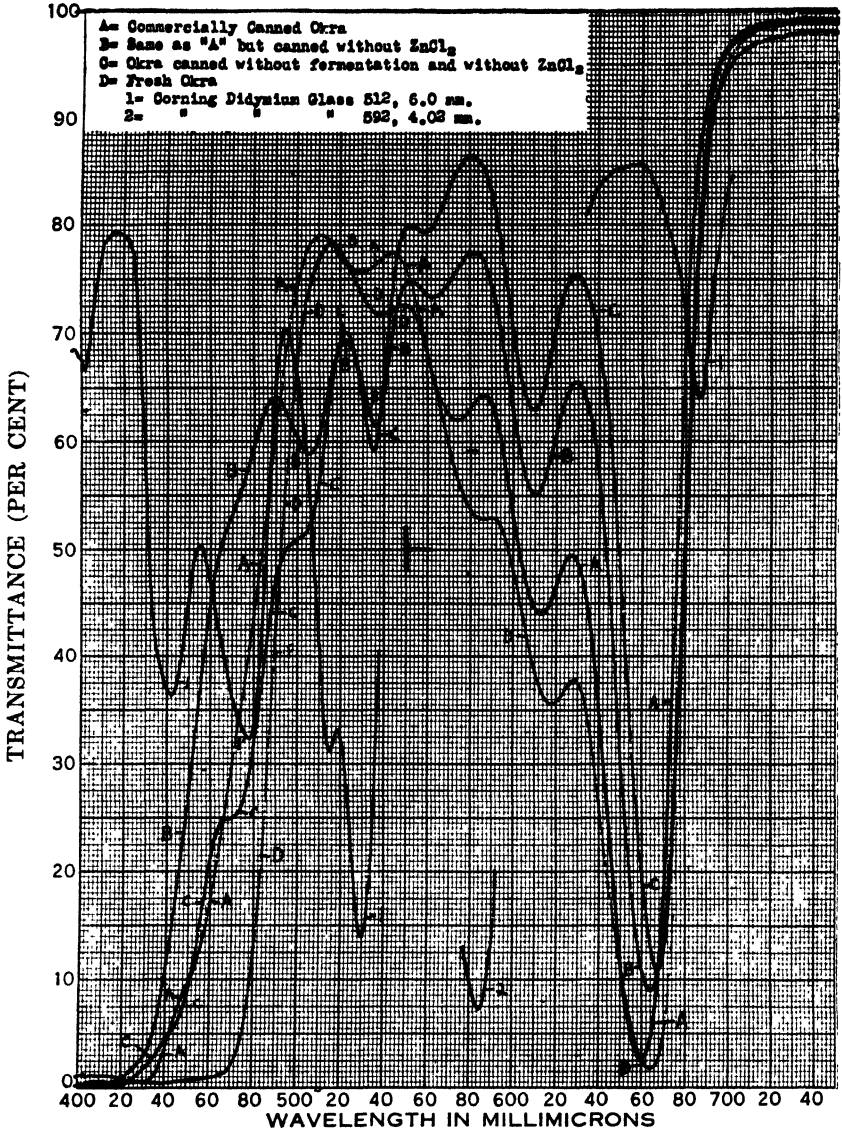


FIG. 1.—CRUDE ETHEREAL EXTRACTS.

TABLE 1.—Crude ethereal extracts (Fig. 1)

SAMPLE	MAJOR ABSORPTION PEAK		MINOR ABSORPTION PEAKS	
	$m\mu$		$m\mu$	
A	658.9		612.6, 573.2, 529.5	
B	662.9		609.6, 561.3, 535.4	
C	666.4		609.1, 557.3, 534.9	
D	662.9		617, 585.2, 538.4	

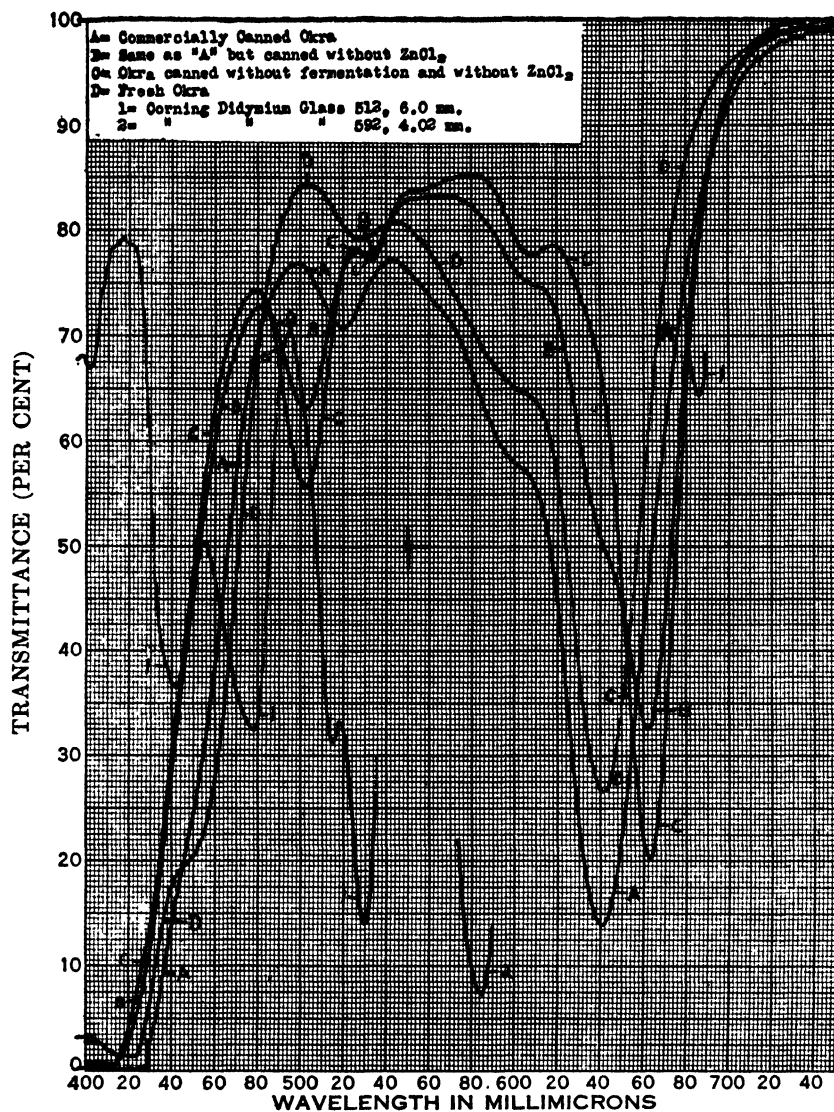


FIG. 2.—COLD SAPONIFICATION PRODUCTS.

TABLE 2.—Cold saponification products (Figs. 2 and 3)

SAMPLE	CONTAINER PRE- HEATED AT 80°C.	MAJOR ABSORPTION PEAK	MINOR ABSORPTION PEAKS
	hours	mμ	mμ
A	0	640.3	519.4
A <sub>1</sub>	16	639.5	518.5
B	0	661.3	530.5, 502.3
C	0	661.8	607.7, 532.4, 502.3
D	0	640.8	525.5

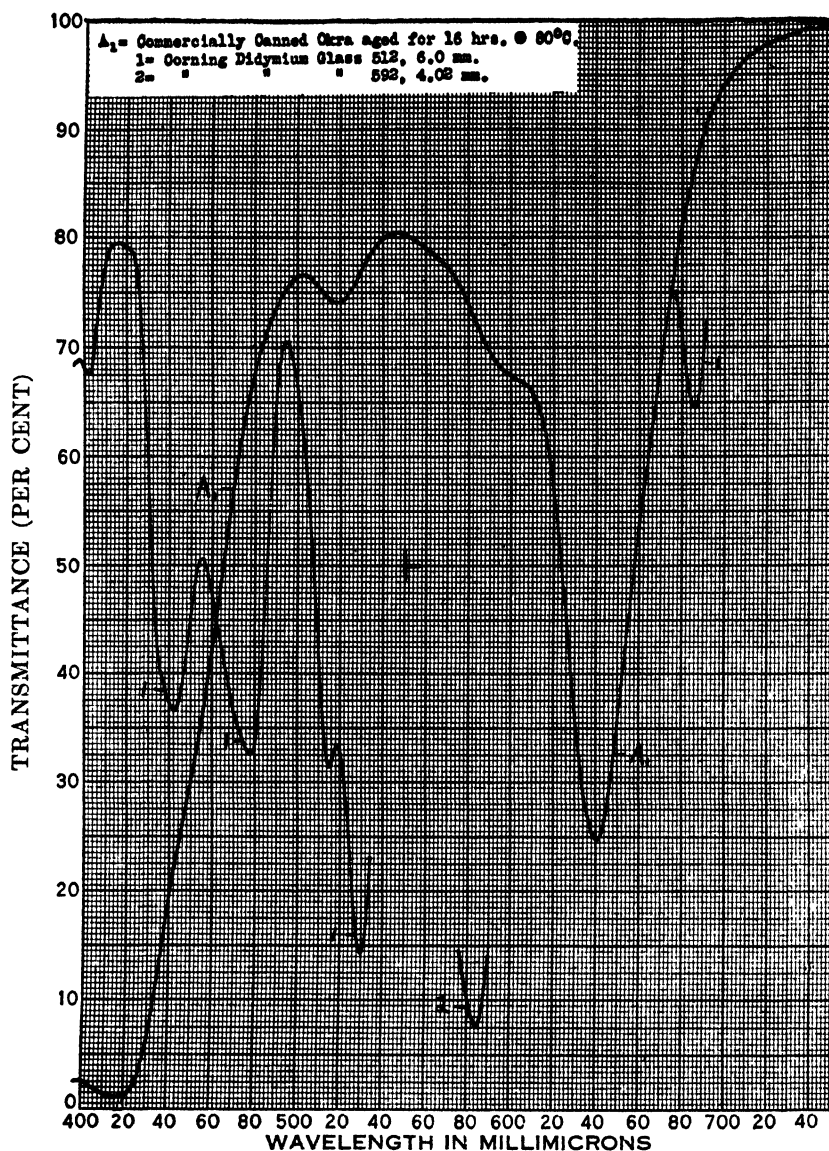


FIG. 3.—COLD SAPONIFICATION PRODUCTS.

the natural green coloring matter has undergone a chemical change. In the opinion of the writers the color molecule has lost its magnesium and combined with zinc. It is this zinc pigment that is responsible for the spectrophotometric shift of the cold saponification products, as well as for the bright green color of ordinary canned okra. Furthermore, this new color is much more stable towards heat and acid media than the naturally occurring pigment. Yet, the spectrophotometric data indicate that there

is a close chemical relationship between the pigments in the fresh and zinc processed okra.

Aside from the spectrophotometric results it is apparent from the visual examination of Samples B and C (fermented and unfermented okra packed without zinc chloride) that the original green pigment is destroyed during the canning process. Only in the presence of the zinc chloride did the resultant canned okra exhibit a bright green pigment. The processing during the canning procedure destroys the natural pigment and the decomposition products react with the zinc chloride to synthesize a stable colored compound.<sup>3</sup>

This work has suggested a number of interesting problems. One of the writers (H. F.) is now engaged in analyzing the green pigment of fresh and canned okra for organically combined zinc, and later investigations will be conducted on the optimum pH as well as on the amount of zinc salts necessary for the synthesis of the new pigment.

#### SUMMARY

Transmission curves in the spectral region of 400–750 m $\mu$  have been obtained for the crude ethereal extract and the cold saponification products of the green pigment in fresh okra, canned fermented okra, canned unfermented okra, and canned fermented okra treated with zinc salts.

The spectrophotometric data indicate that the addition of zinc salts to canned okra results in the synthesis of a pigment containing zinc. This new color is very similar chemically to the naturally occurring chlorophyll that it replaces. The work is being continued.

#### ACKNOWLEDGMENT

The writers wish to express their appreciation to V. B. Bonney of the Food and Drug Administration for his interest and helpful suggestions during the course of this study.

#### MICRODETERMINATIONS FOR ORGANICALLY COMBINED METALS IN PIGMENT OF OKRA\*

By H. FISCHBACH (U. S. Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

In another paper Fischbach and Newburger (see p. 134) conclude as a result of spectrophotometric studies that the green pigment in canned okra, packed with the addition of small quantities of zinc chloride, is not the natural pigment, chlorophyll, found in fresh okra. To verify this conclusion the writer undertook to investigate the organically combined metals in the extracted pigment of fresh and canned okra.

<sup>3</sup> Willstätter and Stoll, "Investigations on Chlorophyll," p. 294.

\* The work reported in this paper was done because of its bearing on standards of identity for foods.



It is known that the ash of chlorophyll is pure magnesia.<sup>1</sup> Bright green compounds are formed when copper and zinc are introduced into chlorophyll derivatives that have been formed by the elimination of magnesium.<sup>1</sup> Iron may similarly be introduced, but not so readily as the former two metals. Blair and Ayres<sup>2</sup> have demonstrated in the case of peas that pigment magnesium is lost in its entirety during the canning process. Consequently the analyses discussed here are confined to determinations of magnesium, zinc, copper, and iron.

#### HISTORY OF RAW MATERIALS

In the usual commercial method of canning, raw okra is allowed to ferment overnight in a warm brine solution contained in a wooden vat. The vegetable is then drained, trimmed, sorted, blanched, cut into sections, placed in cans, surrounded with a weak solution of zinc chloride, sealed, and processed. Three packs of canned okra, identified as "A," "B," and "C" were prepared at the same plant.

*Sample A* was prepared by the above method. The color of the canned okra was "Apple Green" (Plate XVII)<sup>3</sup> and the pH was 4.8.

*Sample B* was prepared similarly to *Sample A* except that the packing medium was plain water. The color of the canned okra was "Grape Green" (Plate XLI)<sup>3</sup> with occasional pieces resembling "Apple Green" (Plate XVII).<sup>3</sup> The pH was 5.35 (see note).

*Sample C* was not subjected to any treatment in brine solution but was blanched, cut, packed in plain water, and processed. The color of the canned okra was "Dark Olive Buff" to "Citrine Drab" (Plate XL).<sup>3</sup> The pH was 5.75.

*Samples D and D<sub>1</sub>* were fresh okra purchased on the Washington, D. C., market on two separate occasions. Its natural color was "Mineral Green" (Plate XVIII).<sup>3</sup>

Additional commercial packs of okra from three different canners were obtained and identified as "E," "F," and "G." Each of these products had a pH of 4.8.

*Sample E.* The color of the canned okra was "Forest Green" (Plate XVII).<sup>3</sup>

*Sample F.* The color of the canned okra was "Apple Green" (Plate XVII).<sup>3</sup>

*Sample G.* The color of the canned okra was "Mineral Green" (Plate XVIII).<sup>3</sup>

NOTE: The occasional bright green pieces of okra observed in *Sample B* might be attributed to small amounts of zinc taken up from the galvanized equipment in the canning plant.

#### PROCEDURE

Remove the seeds and cores from 50 grams of fresh okra (or drained okra), weigh the fleshy, green portion obtained, and wash on a 4 inch Büchner funnel with 100 ml. of distilled water and 100 ml. of double distilled water. Draw air through the funnel for 5 minutes. Transfer to a Waring blender, add 60 ml. of c.p. acetone, and stir at high speed for 2 minutes. Filter on 4 inch Büchner, return residue to blender, add 50 ml. of absolute methanol, and stir for 1 minute at high speed. Filter, and wash the blender and residue with three successive 10 ml. portions of methanol. Transfer the acetone and methanol extracts to a 500 ml. Pyrex separatory funnel, add 75 ml. of ethyl ether, together with 150 ml. of an aqueous 4% NaCl solution,

<sup>1</sup> Willstätter and Stoll, "Investigations on Chlorophyll," pp. 130 and 294.

<sup>2</sup> *Ind. Eng. Chem.*, 35, 86 (1943).

<sup>3</sup> Ridgeway "Color Standards and Nomenclature" (1912).

and shake vigorously. Draw off the aqueous phase and shake with 25 ml. of ether. Transfer the combined ethereal phases to a 250 ml. Pyrex separatory funnel, add 100 ml. of the 4% NaCl solution, and shake. Discard the aqueous phase and shake the ethereal phase with another 100 ml. portion of brine solution. Discard aqueous phase, add 1-2 ml. of methanol for a sharp interface, and discard the residual aqueous phase. Transfer the ethereal phase to a tared 150 ml. beaker, evaporate on steam bath almost to dryness, and dry overnight in the presence of  $P_2O_5$ . Weigh the dried pigment, add 1 ml. of redistilled  $HNO_3$ , and evaporate to dryness on a hot plate. Add 1 ml. of 18 N  $H_2SO_4$ , evaporate to copious fumes, add dropwise 1-2 ml. of redistilled  $HNO_3$ , and continue heating until the excess  $HNO_3$  is removed. Cool, dilute with 10 ml. of double distilled water, and evaporate to incipient fumes. Transfer to a 25 ml. volumetric flask,† neutralize to methyl orange with distilled  $NH_4OH$ , add 1 ml. of normal HCl (made from distilled HCl), and make up to volume.

Analyze a 5 ml. aliquot for zinc by the photometric dithizone method of Cowling-Miller,<sup>4</sup> using a neutral wedge photometer,<sup>5</sup>  $\frac{1}{2}$  inch cell, and filter No. 51 (510  $m\mu$ ).

Determine copper by the Greenleaf<sup>6</sup> method, using the  $CCl_4$  solution of metal dithizonates obtained in the Cowling-Miller method (second extraction) after the zinc has been removed by shaking with 50 ml. of 0.02 N HCl. Proceed as directed in *This Journal*, 25, 390, from line 18 of the section "Isolation of Copper," beginning with the sentence "To the dithizone solution add 10 ml. of the 5%  $H_2SO_4$  and 0.5 ml. of bromine water. . . ."

Determine iron and magnesium in the aqueous phase, which is discarded in the Cowling-Miller<sup>4</sup> method (end of the second paragraph under heading "First Extraction"). Evaporate almost to dryness in platinum, add 1 ml. of 18 N  $H_2SO_4$ , warm, evaporate to dryness, and ash in an electric muffle at 500°C. for 1 hour; cool, add 1 ml. of 18 N  $H_2SO_4$ , warm, transfer to a 25 ml. graduated flask, and make up to volume. Analyze a 5 ml. aliquot for magnesium by the titan yellow method of Koltoff.<sup>7</sup>

To a 10 ml. aliquot of the above solution add 1 ml. of 7.2 N  $NH_4OH$  and determine iron by the use of *o*-phenanthroline, as described by V. E. Munsey,<sup>8</sup> employing a 4 inch cell filter No. 51 (510  $m\mu$ ) and neutral wedge photometer.

NOTES: The method of Blair and Ayres was followed for the pigment extraction with minor variations to introduce the use of the Waring blender into the method. To prevent the "tannates" from precipitating,  $Na_2CO_3$  was not used in the 4% NaCl solution.

The dried, extracted pigment essentially consists of the green and yellow pigments, their decomposition products, and some waxes.

The drained weight of the canned okra was obtained by placing the product in a perforated basket of an international chemical centrifuge and the liquid was extruded by washing (ca. 1000 ml. of tap water) and centrifuging alternately for 5 minutes.

The preparation of the double distilled water and distilled reagents was done in Pyrex.

The subdivisions of each canned sample were separate cans, and these were analyzed at different times. The subdivisions of Samples D and of  $D_1$  were separate portions taken from each of these two lots of fresh okra. A blank of all reagents was included with each set of analyses.

† When fresh okra was the raw material, the solution was made up to volume at this point and a 5 ml aliquot was analysed for magnesium by the colorimetric method of Koltoff.<sup>7</sup> This procedure expedites the analysis when it is known that organically combined Cu, Zn, and Fe are absent in the pigment.

<sup>4</sup> *Ind. Eng. Chem.*, 13, 145 (1941).

<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 13, 218 (1940).

<sup>6</sup> *This Journal*, 25, 385 (1942).

<sup>7</sup> *Chem. Weekblad*, 24, 254 (1927); *Biochem. Z.*, 185, 344 (1927).

<sup>8</sup> To be published.

TABLE 1

SAMPLE	SUB-DIVISION	NET WT.	DRAINED WT.	WT. FLESHY PORTION FROM 50 GRAMS OF DRAINED OKRA	WT. OF DRIED PIGMENT	METALS FOUND IN THE PIGMENT FROM 50 GRAMS OF DRAINED OKRA (MICROGRAMS)			
						Zn	Cu	Fe	Mg
A	1	ounces 19.52	ounces 7.57	grams 21.68	grams 0.0630	44.3	0	0	0
	2	19.47	6.00	21.41	0.0629	43.9	0	0	0
B	1	19.52	8.02	26.71	0.0570	4.9	0	0	0
	2	19.52	7.67	20.26	0.0610	6.1	0	0	0
C	1	19.98	8.08	26.62	0.0668	0	0	0	0
	2	19.84	6.51	17.96	0.0630	0	0	0	0
D	1			25.00*	0.0566	0	0	0	99.5
	2			26.00*	0.0637	0	0	0	101.3
D <sub>1</sub>	1			24.00*	0.0672	0	0	0	119.5
	2			25.50*	0.0590	0	0	0	114.0
E	1	20.00	9.00	28.96	0.0805	26.4	0	0	0
	2	20.12	8.95	25.60	0.0746	24.9	0	0	0
F	1	29.30	8.81	32.04	0.0824	48.3	0	0	0
	2	29.27	9.93	29.25	0.0902	52.1	0	0	0
G	1	19.87	7.76	31.17	0.0990	89.9	0	0	0
	2	19.97	6.52	22.85	0.1026	95.6	0	0	0

\* Weight of okra flesh obtained from 50 gms. of the original fresh okra.

### DISCUSSION

It is apparent from Table 1 that magnesium is present only in the pigment of fresh okra. On the assumption that 2.7 per cent of pure magnesium is present in chlorophyll<sup>1</sup> the data indicate a range of 75-85 p.p.m. of chlorophyll for relatively fresh okra. Since magnesium is a part of the chlorophyll molecule it is obvious that no chlorophyll exists in any of the canned samples. Yet, a bright green coloration was present in all of the canned samples except Sample C, which had not been fermented nor packed with zinc chloride. Sample B (fermented but packed without zinc

chloride) exhibited bright green coloration only in occasional pieces. Correspondingly smaller amounts of zinc were found in the pigment of this sample. It is probable that fermentation permits the okra to absorb the small quantities of zinc from the galvanized canning equipment. The other canned samples exhibited significant quantities of pigment zinc. In agreement with visual examinations the greatest quantities of pigment zinc were found in Sample G, which displayed the greatest intensity of bright green.

In no instance was copper or iron detected in the pigment. Consequently, the green color observed in commercially canned okra can be attributed only to the synthesis of a green organic zinc compound, not natural to okra.

One can only conjecture as to the differences in the amount of pigment zinc found in the canned okra of different canneries. Further work is planned for some future date.

#### SUMMARY

Microdeterminations were made for organically combined magnesium, iron, copper, and zinc in the pigment of fresh and canned okra. Magnesium was found only in the pigment of fresh okra; no other metals were present. No metal was found in the pigment of the okra that had been canned without fermentation. Zinc was the only metal found in the pigment of the okra canned after slight fermentation.

The green color in commercially canned okra is due to a synthesized compound in which zinc is organically combined.

#### ACKNOWLEDGMENT

The writer wishes to express his appreciation to V. B. Bonney, V. E. Munsey, and A. K. Klein, of the Food and Drug Administration, for their interest and helpful suggestions during the course of this study.

### REFRACTIVE INDICES OF INVERT SUGAR SOLUTIONS AND THE CORRECTION FACTOR OF DE WHALLEY

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.)

De Whalley<sup>1</sup> determined the refractive indices of invert sugar solutions prepared by mixing equimolecular quantities of dextrose and levulose, and expressed the results in terms of the concentration of sucrose solutions. It was found that the sucrose values obtained in this manner are lower than the actual concentrations of the invert sugar solutions, and that they must be corrected by multiplying the percentage of invert sugar by a

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<sup>1</sup> *Intern. Sugar J.*, 37, 353 (1935); see also C. F. Snyder, *This Journal*, 19, 399 (1936).

factor and adding the product to the sucrose concentration observed with the refractometer. For solutions containing invert sugar alone the correction factor was found to average 0.008, but for partially inverted sucrose sirups prepared with invertase the factor averaged 0.022 and for sirups prepared by inversion with acid the average factor was 0.021. This seeming inconsistency prompted a closer study of de Whalley's original data.

#### INVERT SUGAR ALONE

As a first step, the refractometer readings obtained by de Whalley for equimolecular mixtures of dextrose and levulose, recorded by him as per cent sucrose, were converted into refractive indices. From the results the following two equations were computed:

$$n_D^{20} = 1.33299 + 0.0014208p + 0.0000056297p^2 \quad (1)$$

for the range from 0 to 45.67 per cent invert sugar, and

$$n_D^{20} = 1.33027 + 0.0015020p + 0.000005305p^2 \quad (2)$$

for the range from 39.54 to 66.72 per cent invert sugar.

The values found experimentally by de Whalley are compared in Table 1 with those calculated from the above two equations.

The same equations, (1) and (2), give the refractive indices of invert sugar solutions, in steps of 5 per cent concentration, shown in Table 2. The equivalent percentages of sucrose are also given, and in the last column the corresponding correction factors.

TABLE 1.—*Refractive indices of invert sugar solutions, calculated versus found*

INVERT SUGAR	$n_D^{20}$ FOUND	$n_D^{20}$ CALCULATED EQUATION 1	DIFF. $\times 10^4$	$n_D^{20}$ CALCULATED EQUATION 2	DIFF. $\times 10^4$
<i>per cent</i>					
9.61	1.3471	1.3472	+1		
14.22	1.3544	1.3543	-1		
15.10	1.3558	1.3557	-1		
18.74	1.3617	1.3616	-1		
20.11	1.3638	1.3638	0		
24.63	1.3714	1.3714	0		
29.69	1.3800	1.3801	+1		
31.39	1.3832	1.3831	-1		
33.09	1.3861	1.3862	+1		
34.94	1.3895	1.3895	0		
39.54	1.3981	1.3980	-1	1.3980	-1
45.67	1.4098	1.4096	-2	1.4099	+1
55.13	1.4292			1.4292	0
66.72	1.4541			1.4541	0

The figures for 70 to 85 per cent invert sugar were obtained by extrapolation and are shown in parentheses. Some experiments carried out in this laboratory indicate that the refractive indices in this range increase more rapidly than appears from the table, and that the correction factors are really higher than those given.

Table 2 clearly proves that the correction factor is not constant but

TABLE 2.—*Refractive index table for invert sugar solutions based on de Whalley's data*

INVERT SUGAR	$n_D^{20}$	CORRESPONDING SUCROSE	CORRECTION FACTOR
per cent		per cent	
5	1.3402	4.97	0.006
10	1.3478	9.98	0.002
15	1.3556	14.95	0.003
20	1.3637	19.92	0.004
25	1.3720	24.82	0.007
30	1.3807	29.78	0.007
35	1.3896	34.70	0.009
40	1.3988	39.55	0.011
45	1.4085	44.45	0.012
50	1.4186	49.35	0.013
55	1.4289	54.20	0.015
60	1.4395	58.95	0.018
65	1.4503	63.75	0.019
(70)	(1.4614)	(68.45)	(0.022)
(75)	(1.4728)	(73.13)	(0.025)
(80)	(1.4844)	(77.75)	(0.028)
(85)	(1.4963)	(82.35)	(0.031)

steadily increases with the concentration. The increase is noted also in de Whalley's original tables, but this fact was not stressed by him.

#### MIXTURES OF INVERT SUGAR AND SUCROSE

The procedure for computing the refractive indices of solutions containing mixtures of sucrose and invert sugar from those of the components may be illustrated by an example from de Whalley's sucrose solutions partially inverted with invertase. Assuming first that the refractive index is a simple additive constant, the index of a solution containing 22.4 per cent invert sugar, 38.42 per cent sucrose, 60.82 per cent total solids, would be calculated as follows: 22.4 per cent invert sugar would give a refractive index of 1.3676, or an increase of 0.0346 over that of water; 38.42 per cent sucrose would give a refractive index of 1.3966, or an increase of 0.0636 over that of water. The refractive index of the mixture would be  $1.3330 + 0.0346 + 0.0636 = 1.4312$ , against 1.4427 observed by de Whalley, or 0.0115 too low.

It has been shown by Browne<sup>2</sup> that the physical constants of aqueous solutions containing mixtures of solutes are governed by the water concentration, and not by the partial concentrations of the solutes. This rule has been found by Vosburgh to apply to the specific rotation, and by Schwartz with respect to copper reducing power. If the refractive index of the mixture mentioned above is calculated by this rule, it is found that 60.82 per cent invert sugar would give a refractive index of 1.4412, or an

TABLE 3.—*Correction factors for mixtures of sucrose and invert sugar*

INVERT SUGAR	SUCROSE	TOTAL SUGARS	$n_D$	EQUIV. SUCROSE	CORRECTION FACTOR
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>	
30	10	40	1.3990	39.67	0.011
20	20	40	1.3993	39.80	0.010
10	30	40	1.3995	39.90	0.010
					0.010
40	10	50	1.4189	49.50	0.013
30	20	50	1.4192	49.60	0.013
20	30	50	1.4194	49.70	0.015
10	40	50	1.4197	49.85	0.015
					0.014
50	10	60	1.4399	59.15	0.017
40	20	60	1.4403	59.30	0.018
30	30	60	1.4407	59.50	0.017
20	40	60	1.4410	59.65	0.018
10	50	60	1.4414	59.80	0.020
					0.018

increase of 0.1082 over that of water. In proportion, 22.4 per cent of invert sugar would give an increase of  $0.1082 \times 22.4 \div 60.82 = 0.0399$ . Similarly, 60.82 per cent sucrose would give a refractive index of 1.4437, or an increase of 0.1107 over that of water. In proportion, 38.42 per cent sucrose would give an increase of  $1.1107 \times 38.42 \div 60.82 = 0.0699$ . The refractive index of the mixture is  $1.3330 + 0.0399 + 0.0699 = 1.4428$ , against 1.4427 found. This, and other slight deviations of the calculated from the observed values for de Whalley's sucrose solutions inverted with invertase will be further discussed in connection with the data presented in Table 4.

It is now possible to calculate the correction factor for mixtures of sucrose and invert sugar, for any concentration of total sugars within the limits of equations (1) and (2), and for any proportion between invert sugar and sucrose. A few examples are given in Table 3.

<sup>2</sup> *Louisiana Planter*, 67, 44 (1921).

It is noted that the individual correction factors show little variation at each concentration and that their averages check closely with those given in Table 2 for invert sugar alone. The agreement would be even better if the refractive indices of both sucrose and invert sugar were known to the fifth decimal place and the equivalent sucrose concentration could be found more accurately. It would then also be possible to calculate correction factors for mixtures containing very small or very large percentages of invert sugar; for sucrose itself the correction factor of course equals zero.

#### INVERTED SUCROSE SOLUTIONS

De Whalley's experiments upon sucrose solutions heated with invertase for various lengths of time are recorded in Table 4. The theoretical total solids are shown in Column 1; the components of the mixtures are shown in Columns 2 and 3, and it is assumed that they consisted only of invert

TABLE 4.—*Refractive indices of inverted sucrose solutions*

1	2	3	4	5	6	7	8	9
TOTAL SOLIDS	INVERT SUGAR	SUCROSE	$n_D$ CALC'D	EQUIV. SUCROSE	CORRECTION FACTOR	$n_D$ FOUND	EQUIV. SUCROSE	CORRECTION FACTOR
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>			<i>per cent</i>	
60.50	16.0	44.50	1.4423	60.20	0.019	1.4420	60.10	0.025
60.61	18.3	42.31	1.4424	60.25	0.020	1.4420	60.10	0.028
60.82	22.4	38.42	1.4428	60.45	0.017	1.4427	60.40	0.019
60.82	22.4	38.42	1.4428	60.45	0.017	0.4427	60.40	0.019
62.23	50.7	11.53	1.4448	61.30	0.018	0.4446	61.20	0.021
62.30	53.8	8.50	1.4448	61.30	0.019	1.4446	61.20	0.022
62.32	52.4	9.92	1.4450	61.40	0.018	1.4446	61.20	0.021
62.50	56.0	6.50	1.4452	61.47	0.018	1.4446	61.20	0.023
62.51	56.2	6.31	1.4452	61.47	0.019	1.4446	61.20	0.023
62.53	56.7	5.83	1.4452	61.47	0.019	1.4446	61.20	0.023
62.72	60.4	2.32	1.4455	61.60	0.019	1.4453	61.50	0.020
62.76	61.2	1.56	1.4455	61.60	0.019	1.4453	61.50	0.021
					0.0185			0.022

sugar and sucrose. The refractive indices that the solutions should show on the basis of this assumption are found in Column 4, the equivalent sucrose concentrations in Column 5, and the corresponding correction factors in Column 6. Columns 7 to 9 show, in the same order as Columns 4 to 6, the values found experimentally by de Whalley.

The differences between the calculated and found refractive indices are small, and may be ascribed, at least in part, to experimental error. It is significant, however, that they are all in the same direction, and the conclusion appears justified that the solutions prepared by de Whalley contained some impurity that has a refractive index different from the mixture of the pure sugars, or that changes the concentration of the total



solids calculated from the degree of inversion, or both. It is very likely that the long heating of the solution at 46°C., especially in the presence of impurities derived from the added invertase, caused a slight destruction of invert sugar with the consequent formation of small quantities of reversion and other condensation products. This conclusion is further strengthened by the fact that the differences between the calculated and found sucrose equivalents average only 0.09 in the first four mixtures, which were heated for 5 hours or less, but 0.18 in the other eight mixtures, which were heated for 23 hours or more.

The calculated correction factors (Column 6) agree with those given in Table 2 for a total sugar concentration between 60 and 65. But the values found by de Whalley are appreciably higher, since small differences between the calculated and found refractive indices have a great effect on the correction factor. The choice of the proper factor for commercial invert sirups is discussed below.

The concentration of the sirups that de Whalley prepared by inversion with hydrochloric acid is in every case beyond the range for which he determined the refractive indices of invert sugar solutions, and an interpretation of his results is further complicated by the presence of hydrochloric acid in the solutions. When calculations similar to those shown in Table 4 are made for these sirups, and the extrapolated refractive indices given in Table 2 are used, it is found that the values observed by de Whalley are not lower, but higher than those calculated. This only proves that the extrapolated indices in Table 2 are too low. Since the actual values are not known it is impossible to interpret the figures found by de Whalley for this type of sirup.

Nevertheless, useful conclusions may be drawn from de Whalley's findings when they are considered in connection with similar results obtained by Schneller.<sup>3</sup> It is important to note that the correction factors recorded by de Whalley for the sirups inverted with hydrochloric acid vary considerably, from 0.0160 to 0.0236, when the concentrations of total solids were within the narrow range of 72.90 to 75.27. This would indicate that even slight differences in the inversion procedure, not intended or noticed by the experimenter, have a great effect on the correction factor. This may be seen even better from the results of Schneller, who reports three series of experiments. In all of these the sucrose solutions were heated with less than 0.01 per cent of concentrated hydrochloric acid in a boiling water bath. In the first two series, which differ merely in the amount of sucrose taken, the heating was continued until inversion was practically complete, while in the third the heating time was varied so as to obtain differing degrees of inversion. The de Whalley correction factors for Schneller's experiments were calculated by the writer, and the results are summarized in Table 5.

<sup>3</sup> *This Journal*, 9, 156 (1926).

In Series 1 and 2, as well as in Series 3, the average correction factors increase with the concentration, as would be expected. De Whalley, in one experiment with a total solids content of 84.05, found a factor of 0.0261. Although the individual correction factors in Schneller's work varied from 0.0161 to 0.0287, the average, 0.0221, is very close to de Whalley's average factor, 0.0210.

In the manufacture of technical invert sirups, with either invertase or acid and the application of heat, the exact procedure used is bound to vary within rather wide limits from factory to factory, and to a certain

TABLE 5.—*Correction factors for Schneller's inverted sucrose solutions*

SERIES	NUMBER OF SOLUTIONS	RANGE OF TOTAL SOLIDS	AVERAGE CORRECTION FACTOR
1 and 2	3	51.37–57.12	0.0198
1 and 2	5	60.27–68.50	0.0219
1 and 2	5	73.12–78.85	0.0234
3	6	64.0–73.65	0.0210
3	15	77.27–79.10	0.0224
3	1	81.92	0.0242
35*		51.37–81.92	0.0221

\* Three of Schneller's 38 experiments are omitted because of apparent errors in the table.

extent also from batch to batch. The correction factor will vary accordingly. If the refractometer is employed for the determination of the total solids in such products it will be best to use the average correction factor 0.022, based on de Whalley's and Schneller's work. This will compensate for individual deviations from the average in regulatory analyses where samples from many different manufacturers are tested. In individual factories the correction factor can, of course, be evaluated by comparison with dry substance determinations.

A complication arises in the case of high-test molasses, which usually contain several per cent of ash. The writer<sup>4</sup> has shown in a previous paper that when the correction factor 0.022 is used in the determination of total solids in these products by the refractometer the results average slightly higher than those for the dry substance because the ash present in these products raises the refractive index of the product.

#### SUMMARY AND CONCLUSIONS

A refractive index table for invert sugar solutions has been computed on the basis of de Whalley's experiments, and the refractive indices of solutions containing mixtures of sucrose and invert sugar, with or without impurities, are discussed. It is shown that the de Whalley correction factor

<sup>4</sup> *This Journal*, 25, 763 (1942).

increases with the total solids concentration irrespective of whether invert sugar is present alone or in mixture with sucrose, but that at one and the same concentration of total solids the correction factor is practically constant. The correction factor for inverted sucrose sirups varies within wide limits, depending on the exact inversion procedure, but the average factor 0.022 may safely be used for practical purposes.

## RAPID METHOD OF SAMPLE PREPARATION FOR DETERMINATION OF ARSENIC, COPPER, LEAD, NICOTINE, AND PHENOTHIAZINE IN SPRAY RESIDUES ON APPLES

By JACK E. FAHEY, C. C. CASSIL, and H. W. RUSK (U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine)\*

The chemical study of spray residues as a part of investigations of the efficacy of insecticidal materials, spray mixtures, and spray schedules evolves into the routine analysis of sprayed fruit to determine residue loads. Such residue loads vary greatly in magnitude, but they are generally much greater than those encountered on fruit prepared for market by washing. Because of the frequency of spray applications and the number of treatments used, it is necessary to obtain numerous samples from each plot at frequent intervals. It has also been found desirable to have each sample consist of a number of subsamples for determining the natural variations within individual trees and between trees of a plot.

At the laboratories of the Bureau of Entomology and Plant Quarantine at Vincennes, Ind., and Yakima, Wash., such investigations have been conducted for several years. The number of samples analyzed at each station during the 3½-month growing period ranges from 2500 to 4000 per season. The samples include residues from the three principal insecticides used in codling moth experiments in recent years—lead arsenate, nicotine, and phenothiazine—and from copper fungicides. To facilitate the handling of such a large number of samples, it was desirable to simplify the routine laboratory procedure as much as possible.

Chemical methods for the analysis of spray residues on fruit generally include technics of sample preparation that vary with the type of residue and the specific problem at hand. One possibility of simplification, therefore, seemed to be in the development of a uniform technic that would be applicable to all four residues. The sample-preparation procedures used for the study of spray residues may be divided into three groups, involving solvent stripping, partial digestions, or complete destruction of organic material by acid digestion or ashing. Only solvent stripping is applicable to organic insecticides.

\* The first and third authors are stationed at Vincennes, Ind., and the second is at Yakima, Wash. The authors are indebted to workers in the fruit insect laboratories of this Bureau for applying sprays to fruit used in the recovery tests.

Markwood<sup>1</sup> described a solvent process for the recovery of nicotine residues from apples sprayed with nicotine bentonite, which consists in placing the fruit in a container with a measured quantity of solvent, shaking, recovering the solvent and rinses, and diluting to volume. It is the simplest stripping procedure described in the literature, but much physical labor is

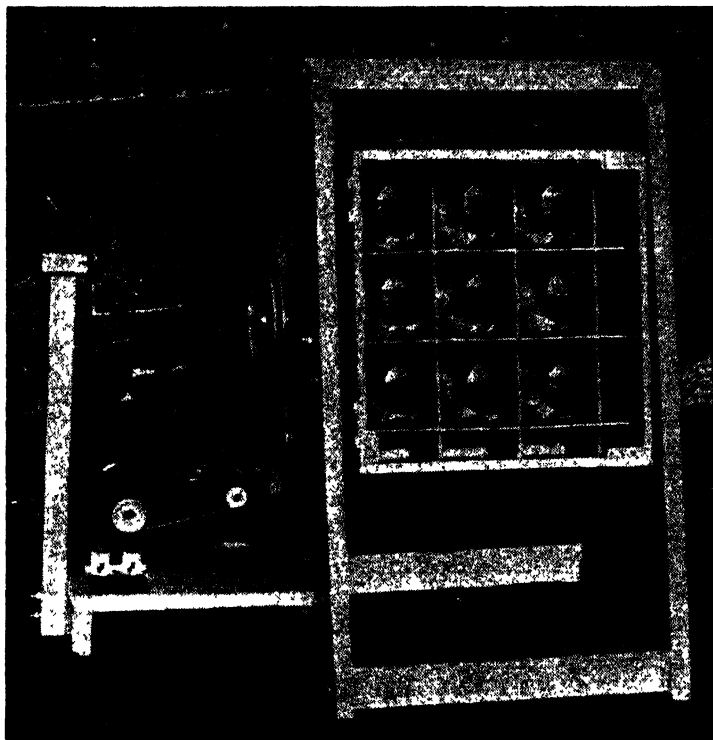


FIG. 1.—SHAKING MACHINE USED IN STRIPPING  
SPRAY RESIDUES FROM FRUIT.

required to shake the samples by hand. The purpose of this paper is to describe a mechanical shaking apparatus and solvents employed with this method for the recovery of lead arsenate, phenothiazine, nicotine, and copper spray residues from sprayed apples and to outline the technic employed in routine laboratory tests.

#### APPARATUS

A study of the action of apples in the extracting jar showed that a tumbling movement caused the fruit surfaces to rub together and also on the wall of the jar; in addition, the fruit was periodically submerged in the

<sup>1</sup> *This Journal*, 22, 427 (1939).

solvent solution and then drained. Such action was more effective in removing visible residue than was mere submerging or whirling of the fruit and solvent. The shaking apparatus was therefore designed to give the sample-containing jars an end-over-end tumbling action.

The shaking machine consists essentially of a revolving cradle, constructed to accommodate sample jars and driven by an electric motor through reducing gears and pulleys. The machine constructed and used at Vincennes is shown in Fig. 1. The parts (except screws, bolts, nails, and electrical wiring) required for its construction are as follows:

Metal	Lumber	
1 Electric motor, $\frac{1}{2}$ H.P.	2 by 4's:	
1 Reducing gear, 50:1 ratio, with $1\frac{1}{2}$ -and 4-inch pulleys	4 pieces	4 $\frac{1}{2}$ feet
1 Pulley, 10-inch	2 pieces	4 feet
1 Pulley, 2-inch	4 pieces	2 $\frac{1}{2}$ feet
2 Belts, 54-inch and 26-inch	2 pieces	2 feet
2 Roller bearings and retainers	1 piece	1 $\frac{1}{2}$ feet
	2 by 2's:	
	1 piece	2 $\frac{1}{2}$ feet
	5-ply veneer:	
	1 piece	20 by 20 inches
	2 pieces	22 $\frac{1}{2}$ by 24 inches
	1 piece	22 $\frac{1}{2}$ by 22 $\frac{1}{2}$ inches
	4 pieces	24 by 24 inches

This particular machine was designed to accommodate from 4 to 48 sample jars, depending upon their size, at one time, and for batch operations. At Yakima, a similar, but smaller, machine is employed; it accommodates from 1 to 12 sample jars at one time and is designed for continuous-flow operations. The heavier machine has been operated at a speed of 20 r.p.m. and the lighter machine up to 60 r.p.m. without causing undue strain on the structural parts. Both machines have proved satisfactory in mechanical operation and in recovery of residue. The specific problem in the laboratory would determine which machine to use.

The jars used are varied according to the number and size of fruit included in the sample for analysis. When loaded into the jar the sample should not fill it to more than three-fourths its height. The jars employed include wide-mouthed fruit jars of pint, quart, and 2 quart sizes, 1 gallon reagent bottles (100 mm. mouth) with plastic top, and 2 $\frac{1}{2}$  gallon pickle jars. Jars of any size or type that will prevent contamination of the sample could be used. Metal covers, unless protected by gaskets, introduce a hazard of sample contamination.

#### EXPERIMENTAL TECHNIC

The apples are preferably clipped from the tree and allowed to drop directly into a tared jar. The jar is then weighed and a measured volume of solvent is added (100–500 ml., depending on the size of the jar and

sample and the amount of residue present, but it seldom exceeds one-fifth of the capacity of the jar). The volume of solvent is also varied so that the aliquot to be taken for the final analysis will contain an amount of residue suitable for the chemical method used. The jar is then sealed and turned end over end in a shaking machine for five minutes, after which the liquid is decanted and an aliquot taken for the final determination. No measurable absorption or other loss of liquid occurs, and it is unnecessary to rinse the fruit or make the strip solution to a known volume.

### SOLVENTS

It is of course necessary to use different solvents for different residues. The following have been found satisfactory:

Residue	Solvent
Nicotine insecticides	0.5% NaOH in water
Phenothiazine	95% ethanol
Lead arsenate	6% NaOH in water
Lead arsenate with lime or bordeaux	5% $\text{NH}_4\text{NO}_3$ plus 5% $\text{HNO}_3$ (water solution)
Copper fungicide	

The final analysis of solutions of spray residue may be made with any standard method that can be adapted. The methods of analysis used at the Vincennes and Yakima laboratories are as follows: Nicotine by the Markwood colorimetric method,<sup>1</sup> phenothiazine by the Eddy method,<sup>2</sup> arsenic by the bromate method<sup>3</sup> or by the rapid titrimetric method described by Cassil,<sup>4</sup> lead by the electrolytic precipitation and thiosulfate titration method,<sup>5</sup> and copper by the technic devised by Rusk.<sup>6</sup>

The choice of solvents for the recovery of nicotine, phenothiazine, and copper residues was based on the recommendations of the writers of the methods employed for final analysis, whereas for stripping lead arsenate residues the choice was made from preliminary laboratory tests with solvents and mixtures previously used for that purpose.

### RECOVERY TESTS

During the 1940 and 1941 growing seasons a number of experiments were conducted to determine the recovery of residues by the stripping process. The samples, after being stripped with appropriate solvent, were rinsed twice with fresh water and treated to recover the residual residue (that remaining after stripping). To recover the residual residues of nicotine and phenothiazine, the fruit was stripped a second time with solutions of 0.5 per cent sodium hydroxide and 95 per cent ethanol, respectively. In 15 observations on nicotine-sprayed fruit and 25 observations

<sup>1</sup> Eddy and De Eds, *Food Res.*, 2, 305 (1937).

<sup>2</sup> Jones, W. C., *This Journal*, 17, 202 (1934).

<sup>3</sup> *Ibid.*, 24, 196 (1941).

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1940, 400.

<sup>5</sup> Rusk, H. W., *This Journal*, 23, 980 (1942).

on phenothiazine-sprayed fruit, it was found to be impossible to recover sufficient compound to indicate that more than a trace remained on the sample after the first stripping.

The recovery of residues present after stripping, from fruit sprayed with lead arsenate and copper fungicides, was accomplished by peeling the fruit; digesting the peel with sulfuric and nitric acids; and determining the copper, lead, or arsenic remaining on the entire sample. The results of these experiments are given in Table 1. The average recovery of lead and

TABLE 1.—*Recovery of arsenious oxide, lead, and copper from spray residues by the stripping process*

MATERIAL RECOVERED	TYPE OF RESIDUE	NUMBER OF OBSERVATIONS	RANGE OF RESIDUE	RECOVERY	STANDARD DEVIATION OF PER CENT RECOVERY
			mg.	per cent	
Arsenious oxide	Lead arsenate-Bordeaux	16	3.2- 10.8	96.9	±0.82
	Lead arsenate	33	5.9-128.0	98.2	±0.92
	Lead arsenate-Bordeaux	12	5.9- 14.8	97.4	±0.84
Lead	Lead arsenate-Bordeaux	14	11.2- 31.6	98.2	±0.89
Copper	Lead arsenate-Bordeaux				
	Nicotine bentonite- copper phosphate	6	7.0- 37.6	97.3	±1.16

arsenious oxide from lead arsenate or lead arsenate-Bordeaux residues ranged from 96.9 to 98.2 per cent, depending upon the type of residue and the solvent used. The standard deviations of these observations are consistently less than  $\pm 1.0$  per cent. The recovery of copper residue averages 97.3 per cent, with a standard deviation of  $\pm 1.16$  per cent.

When fruit sprayed with lead arsenate-copper fungicide mixtures was rinsed after being stripped with ammonium nitrate plus nitric acid and then stripped a second time with the same solvent, the quantities of arsenic and copper recovered by the second stripping were not of sufficient magnitude to measure by the chemical methods employed. The quantity of lead recovered in the second stripping averaged 0.8 per cent of the total, and the percentage recoveries had a standard deviation of 0.34 per cent.

#### ADVANTAGES OF METHOD

1. The apparatus can be constructed at any laboratory at a very low cost.
2. The processes are rapid.
3. The solvent-stripping process introduces little hazard of contamina-

tion of samples and reduces to a minimum the loss of residue from the fruit surface by handling.

4. Where the same stripping solution can be used to recover several residues—such as the ammonium nitrate-nitric acid solution for recovery of lead, arsenic, and copper from lead arsenate-copper fungicide spray residues—all analyses can be made on a single sample, and the labor of sampling and sample preparation thus kept at a minimum.

#### SUMMARY

The problem of analyzing from 2500 to 4000 samples of fruit for spray residue over a period of  $3\frac{1}{2}$  months made it desirable to develop a rapid and uniform preparative technic. It was necessary that this technic be applicable to the analysis of lead, arsenic, copper, nicotine, and phenothiazine in spray residues. An apparatus for the handling of such samples in batch lots or by a continuous-flow process is described. Suitable solvents for the recovery of such residues have been developed or adapted, and experiments with field-sprayed samples show that the recovery of residues by this technic is in excess of 96.9 per cent. That the technic is precise is shown by the fact that the standard deviation of the per cent recovery of residues of lead and arsenious oxide is not greater than  $\pm 1.0$  and with copper residues amounts to  $\pm 1.16$ .

#### SEMIMICRO DETERMINATION OF SILVER

By S. REZNEK (Philadelphia Station, Food and Drug Administration, Federal Security Agency)

Certain types of medicinal preparations containing silver compounds in concentrations of the order of 0.1 per cent, on a silver basis, are packaged in units containing about 2 grams of material, so that each unit contains about 0.002 gram of silver. Since the determination of this amount of silver by either of the usual procedures—the gravimetric chloride or volumetric thiocyanate methods—would require the use of micro equipment, which was not available, a method was worked out which requires no equipment or material beyond that ordinarily found in the analytical laboratory. Also, since organic matter is often present, the method includes a procedure for obtaining the silver in ionic form in a solution free of materials other than sulfates arising from the sulfuric acid used in a wet combustion.

The method involves precipitation of the silver as iodide, which is then converted to silver bromide and iodic acid by the action of bromine, followed by addition of potassium iodide and titration of the liberated iodine. The procedure has the usual advantages of an iodate method, i.e., the 6-fold liberation of iodine and the sharp starch-iodide end point.



## METHOD

Place a sample equivalent to 1–5 mg. of Ag in a 100 ml. Kjeldahl flask, together with a few glass beads to prevent bumping. Add 2.0 ml. of concentrated  $\text{H}_2\text{SO}_4$  and 5 ml. of concentrated  $\text{HNO}_3$ . Digest until oxidation is complete, carefully adding fresh portions of  $\text{HNO}_3$  if necessary. Heat to fumes of  $\text{H}_2\text{SO}_4$  until most of  $\text{HNO}_3$  is driven off. (It is unnecessary to remove the last traces of  $\text{HNO}_3$ .) Cool, dilute with water, and rinse into 150 ml. beaker with small portions of water to a volume of 40–50 ml. (Alternatively, if the type of sample allows, it may be ashed, taken up in dilute  $\text{H}_2\text{SO}_4$ , and the method followed from this point.) Neutralize to methyl red with ammonia water, add 2–3 drops in excess, heat nearly to boiling, and add 0.1% KI solution in 100% excess over the amount calculated to be equivalent to the silver present. Remove from heat, add 10%  $\text{H}_2\text{SO}_4$  until red color of indicator is restored, and then 5 ml. in excess. Let stand 2–3 hours in dark, with occasional stirring.

Filter through a 20 ml. Caldwell (removable bottom) asbestos crucible, transferring precipitate with aid of  $\text{HNO}_3$  (1+100) and removing any particles adhering to the beaker with a rubber policeman. Wash the precipitate and walls of the crucible with several small portions of the dilute  $\text{HNO}_3$  to remove all excess reagent. (Avoid the use of water alone as a wash since this sometimes renders the precipitate colloidal and causes it to run through the filter.) Transfer the removable bottom, with its adhering pad and precipitate, to a 300 ml. Erlenmeyer flask and wash any precipitate adhering to the walls of the crucible into the flask with a small volume of water. Add 40–50 ml. of saturated bromine water, a few drops of liquid bromine (0.2–0.3 ml.), and swirl the contents of the flask until the asbestos pad is thoroughly dispersed. Boil gently, rotating the flask occasionally, until the bromine is largely removed, as shown by the virtual disappearance of the yellow color. Remove from heat, and add, while hot, 1 ml. of 5% phenol to remove the last traces of bromine. Cool under the tap to room temperature, add 10 ml. of 10%  $\text{H}_2\text{SO}_4$  and ca. 0.1 gram of KI, and titrate with 0.01 N thiosulfate, using starch indicator.

1 ml. of 0.01 N thiosulfate = 0.000180 gram of Ag.

## DISCUSSION

The method was suggested by that used by Evans, Hanson, and Glasoe<sup>1</sup> for the determination of small quantities of iodides in the presence of large quantities of bromide. These authors found it necessary to use chlorine water to convert the silver iodide to iodate in the presence of large quantities of silver bromide. Since it is necessary to convert only silver iodide in the present instance, bromine water was used because of its greater convenience, and it was found to convert quantitatively the silver iodide to silver bromide and iodic acid.

The silver iodide is precipitated in slightly ammoniacal solution, followed by acidification, as in the technic described by Hillebrand and Lundell,<sup>2</sup> since this procedure seems to flocculate the precipitate more readily than does direct precipitation in an acid solution.

The precipitated silver iodide is colored pink by adsorbed methyl red but this is apparently destroyed by the subsequent bromine treatment and was not found to interfere.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 314 (1941).

<sup>2</sup> *Applied Inorganic Analysis*, p. 591. John Wiley and Sons, New York (1929).

Treatment with bromine water in the cold results in 85-95 per cent conversions, whereas a few minutes at boiling temperature gives quantitative results. A few drops of liquid bromine are added to maintain a high bromine concentration at the beginning of the treatment. The blank due to the reagents was found to be equivalent to 0.1 ml. of 0.01 *N* thiosulfate. If higher blanks are obtained the bromine should be washed before use by shaking with water.

As shown in the table it is feasible to determine down to about 0.2 mg. of silver. The silver nitrate samples were prepared from dilutions of silver

*Recovery of silver*

TYPE OF MATERIAL	AMOUNT OF SAMPLE	Ag EQUIVALENT	IODATE FOUND, CALCULATED AS Ag	RECOVERY
	<i>ml.</i>	<i>gram</i>	<i>gram</i>	<i>per cent</i>
AgNO <sub>3</sub> Solution	0.2	0.00026	0.000263	101.4
	0.5	0.00065	0.000636	97.9
	1.0	0.00130	0.00127	98.0
	2.0	0.00260	0.00258	99.8
	2.0	0.00260	0.00258	99.8
	5.0	0.00648	0.00643	99.2
	<i>gram</i>			
Ag Picrate Powder <sup>a</sup>	0.0050	0.00158	0.00157	99.4
	0.0050	0.00158	0.00158	100.0
Ag Picrate Jelly <sup>b</sup>	1.875	0.00155	0.00153	98.7
	2.323	0.00192	0.00188	97.9
	2.002	0.00166	0.00165	99.4

<sup>a</sup> Contained 31.6% Ag by gravimetric analysis.

<sup>b</sup> Contained 0.258% Ag picrate, on 32.1% silver basis, by gravimetric assay on large sample.

nitrate standardized by the gravimetric silver chloride method. The silver picrate samples were prepared from a solution of the crystalline material and the "silver picrate jelly" was a water-soluble ointment containing 0.258 per cent of anhydrous silver picrate. The latter two materials were supplied through the courtesy of John Wyeth and Bro., Inc., Philadelphia.

### CONCLUSION

A method is described for determining silver in amounts of the order of 1 mg. in organic or inorganic salts with an error of about 2 per cent. The method involves precipitation of silver as the iodide, which is then converted to silver bromide and iodic acid, and the latter is determined by reaction with iodide followed by thiosulfate titration.

## CAROTENE AND VITAMIN A IN COMMERCIAL BUTTER

By G. S. FRAPS and A. R. KEMMERER (Texas Agricultural  
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A national cooperative project to obtain information relative to vitamin A values in butter was approved by the Directors of the Association of Land Grant Colleges in November 1941. The project was originally recommended by the Committee on Food and Nutrition of the National Research Council, to ascertain the actual variation in the vitamin A values of market butter in towns and cities located in different sections of the United States.

Chemical methods to evaluate the vitamin A potency of commercial butter are needed for the purpose of such an investigation. Several workers (1, 2, 3) have studied the relation between the carotene and spectro vitamin A content of butter fat and its vitamin A potency as measured by biological methods. Fraps, Kemmerer, and Meinke (2) presented an equation showing the relation between the vitamin A potency as measured in U.S.P. units by means of rats and the carotene and spectro vitamin A in butter fat as determined by chemical analysis. This butter was not artificially colored, while commercial butter may be artificially colored.

In one of the papers referred to above (2) the carotene was determined by reading the density at 470 and 480  $m\mu$  in a solution of the butter in petroleum benzin. This method would not be applicable in the presence of appreciable amounts of artificial or other color. The spectro vitamin A was determined by reading the density of absorption at 328  $m\mu$  of a methanol solution of the unsaponifiable residue. This solution contains carotene, and the density read must be corrected for the carotene present. If artificial butter color or other coloring matter other than carotene is present, a different factor for correction is needed.

## EFFECT OF ARTIFICIAL COLORS ON ANALYSIS

According to the methods of the A.O.A.C. (4) certain colors extracted by 90 per cent alcohol from fats will remain in the alcohol when washed with gasoline. These colors—*aniline yellow*, *butter yellow*, *aminoazotoluene*, *auramine*, *sudans*, *yellow OB*, *yellow AB*, etc.—would therefore not affect the determination of either carotene, if the A.O.A.C. method for hay and dried plants (6) was used, or the spectro vitamin A (5). Neither would other colors that are not extracted from alkaline solutions, such as *annatto* (7), interfere. Carotene is being sold as a butter color, but this has vitamin A potency.

Nine commercial butter colors, donated by the manufacturers, were examined to ascertain their effect upon the determination of carotene and spectro vitamin A. Portions were heated with alcoholic potash and extracted with petroleum benzin, and the benzin solution was shaken

with 90 per cent methyl alcohol as directed in the A.O.A.C. method for carotene (6). The results, given in Table 1, show that all the colors are soluble in alcoholic potash, but that three of them are not removed from the alcoholic solution by extraction with either petroleum benzin or ethyl ether. These three colors would not interfere with the determination of carotene by the A.O.A.C. method (6) or with the determination of spectro vitamin A (5). The six colors extracted with petroleum benzin were removed with 90 per cent methanol, and would not interfere with the determination of carotene. Since they are soluble in ethyl ether, they would not interfere with the determination of spectro vitamin A.

The correction of spectro vitamin A to be made for the colors extracted

TABLE 1.—*Properties of commercial butter colors*

NUMBER	TYPE OF BUTTER COLOR	SOLUBLE IN ALCOHOLIC KOH	EXTRACTED FROM ALCOHOLIC KOH WITH PETROLEUM BENZIN	REMOVED FROM PETROLEUM BENZIN WITH 90% METHANOL	REMOVED FROM ALCOHOLIC KOH WITH ETHYL ETHER
59100	Dairy Laboratories	yes	yes	yes	yes
59101	Preservaline Mfg. Co.	yes	yes	yes	yes
59102	Vegetable, Ed. Long Chemical Co.	yes	no		very slight
59103	Philip R. Park	yes	no		no
59104	Annatto, Chr. Hansen's Laboratory	yes	no		no
59105	Chr. Hansen's Laboratory	yes	yes	yes	yes
59106	Maumee Color Company	yes	yes	yes	yes
59107	Golden Churn Laboratories	yes	yes	yes	yes
59108*	Vegetable, Golden Churn Laboratories	yes	yes	yes	yes

\* Only slightly soluble in petroleum benzin.

by ether was ascertained by the following procedure: One-tenth gram of each of the six colors was saponified with about 25 ml. of 20 per cent alcoholic potassium hydroxide, and the color was extracted with ethyl ether by the method used for spectro vitamin A in butter (5). After evaporation of the ether, the color was taken up in absolute methanol and diluted to appropriate volume, and the density was determined at 450 and 328  $m\mu$ . These data are given in Table 2. All six of the colors absorb considerable light at 328  $m\mu$ , the wave length of maximum absorption for vitamin A. The ratio of the absorption at 328  $m\mu$  to the absorption at 450  $m\mu$  is approximately the same for five of the six colors. The ratio for the sixth color is quite different, but since only a very small portion of this color is removed from alcoholic potash by ether, it can be disregarded. The average ratio of the five samples is .52. This factor can be used to correct for absorption at 328  $m\mu$  due to artificial color as shown later.

TABLE 2.—*Absorption of light by artificial butter colors*

NUMBER	DENSITY AT 328 m $\mu$	DENSITY AT 450 m $\mu$	D 328 D 450
59100	.31	.54	0.57
59101	.33	.66	0.50
59105	.35	.67	0.52
59106	.47	.93	0.51
59107	.35	.68	0.51
59108*	.92	1.18	0.78*
Mean			0.52

\* Very little of the pigment is soluble in ether. Ether-soluble portion taken for experimentation omitted from mean.

#### EXAMINATION OF SAMPLES OF COMMERCIAL BUTTER

In order to ascertain which methods would be needed, examination was made of a number of samples of commercial butter fat. Pure carotene and crude carotene were determined by the saponification method described below. The difference between the crude and pure carotene (0.2–0.4 p.p.m.) was so small that it can be disregarded. The non-carotene color, which is the difference between the pure carotene and the total color of the butter fat, was also determined.

The spectro vitamin A was determined by the method previously described (5). The international units per gram were calculated by the formula (2)  $IU = (S - 0.5)4 + 1.7 C$ , in which IU is the international units per gram, S is the spectro vitamin A in parts per million, and C is the pure carotene; 0.5 is pseudo spectro vitamin A, which does not have vitamin A activity, and 4 is a factor to convert pseudo spectro vitamin A to international units. Results of the analyses are given in Table 3.

The non-carotene color determined as described below, ranged from 1.4 to 5.6 p.p.m.; in most of the sample it was below 3 p.p.m. The three samples containing more than 5 p.p.m. of non-carotene color were subjected to further examination. They were saponified, and the color extracted by petroleum benzin and by ethyl ether was determined. The results are given in Table 4. The color dissolved in ethyl ether would affect the determination of spectro vitamin A. The difference between the total color obtained and the pure carotene is total non-carotene color. Most of the non-carotene color is retained by the aqueous alcoholic solution, only 0.1–0.3 p.p.m. of which is extracted by petroleum benzin and 0.5–0.6 p.p.m. by the ethyl ether. The source of this color is not known, but if it is due to artificial color the quantity present in these samples would involve a correction of only 0.35–0.42 p.p.m. of spectro vitamin A. This quantity is negligible compared with the greater errors involved in the calculation of

TABLE 3.—*Vitamin A content of some commercial butter fats*

LABORATORY NUMBER	PURE CAROTENE	SPECTRO VITAMIN A	NON-CAROTENE COLOR	INTERNATIONAL UNITS PER GRAM BUTTER FAT
	p.p.m.	p.p.m.	p.p.m.	
64561	4.2	7.4	5.4	34.7
64562	5.8	10.0	2.9	47.9
64563	5.6	8.8	2.1	42.7
64564	8.7	9.8	2.8	52.0
64565	3.6	7.8	2.1	35.3
64566	5.9	11.1	2.2	52.4
64567	3.0	7.1	1.4	31.5
64568	4.4	9.5	5.6	43.5
64806	6.3	11.9	2.5	56.3
64807	9.1	10.6	2.7	55.9
64808	11.2	10.7	2.4	59.8
64809	10.0	12.3	2.7	64.2
64813	5.6	8.6	2.7	41.9
64814	7.3	11.5	2.7	56.4
64815	3.7	7.3	5.2	33.5
64816	5.7	10.9	3.9	51.3
64817	4.1	8.1	2.0	37.4
64818	7.7	10.1	3.1	51.5
64819	6.8	10.9	3.0	53.2
64820	7.7	11.2	2.9	55.9
64895	10.2	10.7	1.4	58.1

spectro vitamin A and carotene to U.S.P. biological units of vitamin A. However, six of the butter colors examined would be extracted from alcoholic potassium hydroxide by ethyl ether and would affect the analysis, so that any method used must provide for the presence of these colors.

#### METHODS USED FOR ANALYSIS OF BUTTER

To calculate the vitamin A potency from the analysis, it would be necessary to determine the total color, the crude carotene, and the spectro vitamin A. If the difference between the total color and the crude carotene is small (below 3 p.p.m.), it could be disregarded, but if it is large, a further examination would be desirable in order to correct for the effect of such color on the determination of spectro vitamin A. Also, if visible quan-

TABLE 4.—*Distribution of non-carotene color (p.p.m.)*

LABORATORY NUMBER	TOTAL	EXTRACTED BY PETROLEUM BENZINE	EXTRACTED BY ETHYL ETHER
64561	5.4	0.2	0.5
64568	5.6	0.3	0.5
64815	5.2	0.1	0.6

tities of color are removed from the petroleum benzin solution by 90 per cent methanol further examination is desirable.

*Preparation of butter fat.*—One-fourth to 1 pound of butter was melted in an electric oven at 50°–60°C. The casein and water were allowed to settle, and the clear liquid butter was filtered through a fluted filter in the electric oven at the above temperature. The melted fat was placed in a refrigerator to solidify rapidly.

*Total color (as carotene).*—Five grams of butter fat was dissolved in about 50 ml. of petroleum benzin, transferred to a volumetric flask, and made up to volume, and the total color was estimated as carotene with a photoelectric colorimeter. In the work previously reported (2), the density at 470 and 480 m $\mu$  was read in a Bausch and Lomb spectrophotometer.

*Crude carotene.*—Five grams of butter fat was saponified with approximately 100 ml. of 12 per cent alcoholic potassium hydroxide, the carotene was extracted with petroleum benzin and washed with methyl alcohol, and the analysis was completed as directed in the A.O.A.C. method for carotene (6), but with use of the photoelectric colorimeter.

*Pure carotene.*—The method of selective adsorption was used (8). The crude carotene solution (50 ml.) was shaken with 2.5 grams of activated magnesium carbonate, and the color was read in the photoelectric colorimeter.

*Non-carotene color.*—The difference between the total color and the pure carotene is the non-carotene color. The non-carotene color may be due to coloring materials that have been found to occur to a small extent in natural butter, or to artificial color.

*Artificial color soluble in ethyl ether.*—The fat is saponified and extracted as described previously but with ethyl ether, the ethyl ether is made up to volume, and the color is read and calculated to carotene. The amount of crude carotene was subtracted, and the difference is taken to be the artificial color soluble in ethyl ether.

*Spectro vitamin A.*—Spectro vitamin A was determined by the following method (5).

Five grams of butter, in a 300 ml. flask attached to a condenser by a No. 20 interchangeable glass joint, was refluxed in a stream of nitrogen for 30 minutes with 50 ml. of aldehyde-free 12% alcoholic potash. Cork or rubber stoppers were not used because large quantities of substances that absorb light in the same region of the spectrum as vitamin A are dissolved from them by alcohol or ether. Fifty ml. of water was added, and the mixture was cooled to 4°C. and transferred to a liter pear-shaped separatory funnel; 50 ml. of ether, purified by distilling over KOH, was added, and then 150 ml. of cold water was added. The ether layer was drawn off, and the aqueous alcoholic fraction was extracted three more times with 15 ml. portions of ether. The combined ether solutions were washed repeatedly with cold water until free from alkali, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and placed immediately in a 300 ml. flask attached to a Claisen distillation tube by means of a No. 20 interchangeable joint. The side tube of this Claisen tube was connected to an ordinary condenser to which was attached a side-necked flask, connected to a vacuum pump. The ether was distilled off in nitrogen under reduced pressure. The residue

was taken up in as small quantity as possible of hot absolute synthetic methanol, cooled to room temperature, and made up to volume in a 10 ml. flask, and the impurities were crystallized out by cooling for 2 hours at  $-8^{\circ}\text{C}$ . with an ice-salt mixture. The cold solution was filtered, and the spectro vitamin A was determined with the spectrograph.

The absorption spectra were photographed through a Bausch and Lomb medium quartz spectrograph equipped with a photometer reading in density and a silver electrode. To correct for impurities in the reagents a solution of the same reagents made in a manner similar to that of the vitamin A solution was used in the comparison tube. Preliminary photographs were made at several settings of the photometer. If necessary, the solution was diluted, and additional photographs were made at several more photometer settings. The photographs were made at a density between 0.6 and 1.1 at  $328\text{ m}\mu$  with a depth of 2 cm. If the density was too low, the work was repeated with use of 10 gm. of butter and correspondingly larger quantities of the reagents.

The methanol solution of the unsaponifiable matter contained some carotene. Although its maximum absorption is at about  $450\text{ m}\mu$ , carotene absorbs light to some extent at  $328\text{ m}\mu$ . To correct for this absorption, the parts per million of carotene in the solution of unsaponifiable material from the butter being tested was estimated by the A.O.A.C. method of colorimetric comparison against 0.1%  $\text{K}_2\text{Cr}_2\text{O}_7$  (6). For a 2 cm. depth of solution, the correction in density for carotene is approximately 0.03 per 1 p.p.m. of carotene (5).

The spectro vitamin A content in parts per million of the butters was calculated from the corrected density by the method used by Bauman and Steenbock (1). The Beer-Lambert equation ( $E = 1/cd D$ ) was used, in which  $E$  is the extinction coefficient,  $d$  the depth of the absorption cell,  $c$  the concentration of vitamin A, and  $D$  the density of absorption. The value of 1600 of Carr and Jewell was used for  $E$ . Since 1600 is the density of a 1% solution of the pure vitamin A in a 1 cm. absorption cell, a solution containing 1 p.p.m. would have a density of  $1600 \times .0001$ , or 0.16. Thus, a solution in a 1 cm. cell having a density of 1.0 would contain 6.25 p.p.m. of vitamin A. For 5 grams of butter in 10 ml. of solution and in a 2 cm. cell, the absorption density is multiplied by 6.25 to obtain the parts per million of vitamin A.

*Correction for artificial color.*—If the non-carotene color of the butter fat is over 3 p.p.m., artificial color is present in the methanol and correction should be made for it. The artificial color soluble in ethyl ether should be determined. If the artificial color is assumed to be the same as that given in Table 2, the correction for artificial color equivalent to 1 p.p.m. of carotene would be a density of  $2 \times .22 \times .52 = .228$  in a 2 cm. cell, compared with .03 for carotene. After correction already made for carotene has been allowed for, for each 1 p.p.m. of artificial color 0.6 p.p.m. should be deducted from the spectro vitamin A.

*International Units of vitamin A.*—The biological potency in International Units of vitamin A as in the previous publication (2) was calculated by the equation  $\text{IU} = (S - 0.5)4 + 1.7C$ , in which IU is the number of International Units per gram,  $S$  the spectro vitamin A in parts per million, and  $C$  the carotene in parts per million, read from the total color. In commercial butter fat, the pure or crude carotene in parts per million should be used in place of the total color.

The figures given above refer to the butter fat, which can be determined in the commercial butter or the results may be calculated to butter on the assumption that the butter contains 81 per cent butter fat.

## SUMMARY

The presence of butter color gives high values for carotene in butter fat if the color is read without preliminary purification.



Butter colors do not interfere with the determination of carotene if the butter is saponified, but they may interfere in the determination of spectro vitamin A.

Methods for correcting the results for spectro vitamin A for the presence of artificial color are given.

The samples of butter examined did not contain color that would interfere appreciably with the determination of spectro vitamin A, but artificial butter colors that would have this effect are being sold. The results were calculated to U.S.P. units of vitamin A.

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#### SPECTROPHOTOMETRIC DETERMINATION OF LOVIBOND NUMBER IN BROWN LOVIBOND GLASSES SERIES NO. 52, BREWER'S SCALE

By G. F. BEYER (Alcohol Tax Unit, U. S. Treasury Department,  
Washington, D. C.)

In the brewing and distilled spirits industries the color of wort, beer, whiskey, caramel solutions, etc., is recorded or reported and individual products are standardized almost universally in terms of Lovibond number of the brown-colored glasses series No. 52, recommended by the American Society of Brewing Chemists. Some of these standard glasses have been known to vary appreciably in depth of color, although they may have the same number. Probably the most recent investigations in this connection are those by W. T. Greenaway, E. Singruen, and G. D. Thevenot,<sup>1</sup> and those reported by the Committee on Color of the American Society of Brewing Chemists, B. H. Nissen, Chairman, and Stephen Joza.<sup>1</sup>

These investigators have clearly shown by spectral transmission curves of some duplicate glasses in their possession that the amount of light transmitted may vary appreciably. Similar curves of the transmittance of three sets of glasses used in this laboratory show that the same condition exists.

<sup>1</sup> *Proc. 4th Ann. Meet. Am. Soc. Brew. Chemists*, May, 1941.

Correlation of the numbers of the glasses to spectral transmission has not been attempted, except, perhaps, by Greenaway et al.<sup>1</sup> He mentioned that it would be presumptuous at that time to suggest a correlation between a color standard and per cent transmission because of the variation in duplicate glasses and because it would be difficult to suggest the ideal wave length at which to record the transmission.

Nissen and Joza read the color of beer in a photometer, using a green filter, and then in a Lovibond tintometer, and plotted curves. In substantially the same manner the writer used a neutral wedge photometer for reading the color of whiskey, using a blue filter transmitting light at about 460  $m\mu$ .<sup>2</sup>

There are several objections to this visual matching of colors. It is difficult to obtain the same light intensities in both fields of a Lovibond tintometer, and unless this condition can be obtained duplicate matches can not be made. Readings made on a dark day may vary from those made on a bright day. The sensitivity of the eye to certain colors varies with different persons, and also with the same observer, depending on the strain and physiological make-up.

#### EXPERIMENTAL

An attempt was made to correlate spectral transmittance to Lovibond number by using a Coleman D.M., Model 10-S, photoelectric spectrophotometer having a 30  $m\mu$  exit slit. Before any such correlation could be made, however, it was necessary to make a series of spectral transmittance curves of bourbon and rye whiskey, rum, caramel coloring matter, beer, wort (Figs. 2 and 3) and at least one of the standard glasses (Fig. 1) to determine the optimum or, perhaps preferably, the desired wave length at which the readings should be recorded; that is, the wave length at which the considered constituent (in this case the color of the whiskey, beer, etc.) will exert its maximum optical effect with minimum interference.

Considerable difficulty was experienced at first in obtaining or keeping a balance between the spectrophotometer and electrometer when check readings were attempted, owing to the uneven distribution of the color in the glasses; therefore, some mechanical contrivance was necessary to keep the glass slides in the same position each time they are placed in the monochromatic light beam.

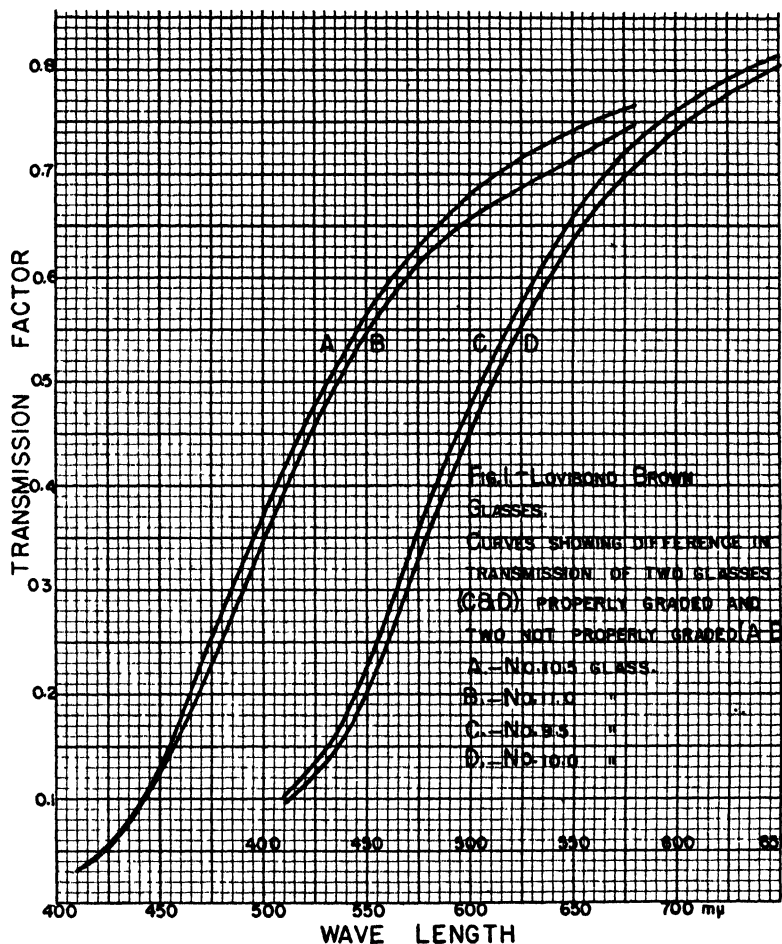
The spectral transmittance curves in Figures 1, 2, and 3 show that the optimum wave length is somewhere in the violet or blue-violet region of the spectrum; that is, between 400 and 460  $m\mu$ . Therefore, a large number of values for T (transmittance) were obtained at 430, 440, 450, 460, and also 500  $m\mu$  by using a 5  $m\mu$  and a 30  $m\mu$  slit and water, air, 50 per cent

<sup>1</sup> *This Journal*, 22, 156 (1939).

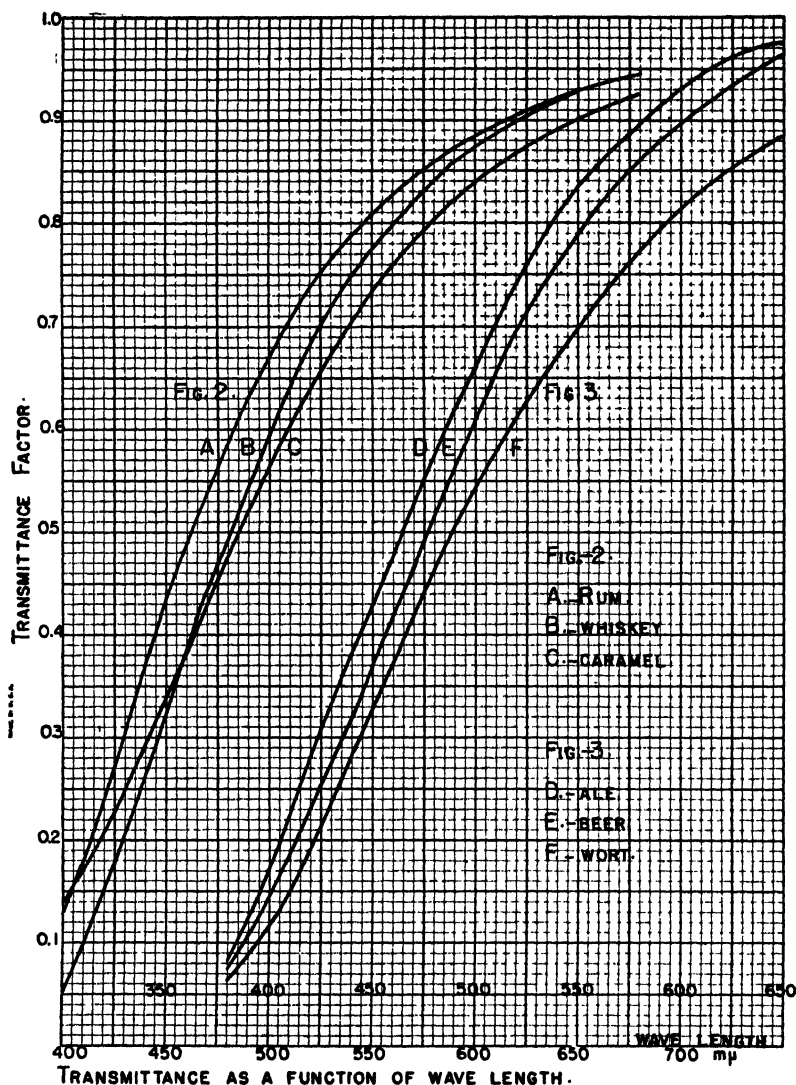
**TABLE 1.—Transmittance (*T*) of Lovibond glasses, series No. 52, at wave length of 430 mμ, showing relationship between Lovibond number, transmittance, and respective densities multiplied by 10**

TRANSMITTANCE VALUES AND RESPECTIVE DENSITIES MULTIPLIED BY 10							
NEW GLASSES				OLD GLASSES			
LOVIBOND NO.	T	—LOG T	—(LOG T)10	LOVIBOND NO.	T	—LOG T	—(LOG T)10
1.0	0.790	0.102	1.0	1.0	0.793	0.102	1.0
1.5	0.711	0.148	1.5	1.0	0.776	0.110	1.1
2.0	0.635	0.197	2.0	1.5	0.711	0.148	1.5
2.5	0.580	0.237	2.4	1.5	0.725	0.140	1.4
3.0	0.500	0.301	3.0	2.0	0.652	0.186	1.9
3.5	0.445	0.352	3.5	2.0	0.634	0.198	2.0
4.0	0.400	0.398	4.0	2.5	0.580	0.237	2.4
4.5	0.365	0.437	4.4	2.5	0.580	0.237	2.4
5.0	0.315	0.502	5.0	3.0	0.506	0.296	3.0
5.5	0.283	0.548	5.5	3.0	0.495	0.305	3.05
6.0	0.248	0.605	6.05	3.5	0.445	0.352	3.5
6.5	0.218	0.661	6.6	3.5	0.463	0.333	3.3
7.0	0.190	0.721	7.2	3.5	0.450	0.347	3.5
7.5	0.173	0.762	7.6	4.0	0.393	0.406	4.1
8.0	0.152	0.818	8.2	4.0	0.388	0.411	4.1
8.5	0.134	0.870	8.7	4.5	0.365	0.437	4.4
9.0	0.114	0.943	9.4	4.5	0.363	0.440	4.4
9.5	0.110	0.959	9.6	4.5	0.365	0.437	4.4
10.0	0.0940	1.027	10.3	5.5	0.283	0.548	5.5
10.5	0.088	1.055	10.55	5.5	0.283	0.548	5.5
11.0	0.075	1.125	11.25	5.5	0.286	0.544	5.4
11.5	0.069	1.161	11.6	6.0	0.247	0.607	6.1
				6.5	0.218	0.661	6.6
				6.5	0.220	0.658	6.6
				6.5	0.222	0.654	6.5
				7.0	0.195	0.710	7.1
				7.0	0.202	0.693	6.9
				7.5	0.173	0.762	7.6
				7.5	0.175	0.757	7.6
				7.5	0.173	0.762	7.6
				8.0	0.146	0.836	8.4
				8.5	0.134	0.870	8.7
				8.5	0.140	0.854	8.5
				8.5	0.135	0.870	8.7
				9.0	0.123	0.910	9.1
				9.0	0.123	0.910	9.1
				9.5	0.110	0.959	9.6
				9.5	0.111	0.955	9.55
				9.5	0.107	0.971	9.7
				10.0	0.100	1.000	10.0
				10.0	0.0990	1.004	10.04
				10.5	0.0850	1.071	10.7
				10.5	0.0800	1.097	10.97
				11.0	0.0705	1.152	11.5
				11.0	0.0790	1.102	11.0
				11.5	0.0675	1.171	11.7
				11.5	0.0725	1.1397	11.4

alcohol, a plain glass slide, and the No. 1 Lovibond glass as references. Concentration-transmittance (C-T) curves were plotted on semilogarithmic graph paper, T as ordinate on the logarithmic scale and C, the number on the Lovibond glasses, as abscissa on the uniform scale, to determine whether or not Beer's law was being followed. The  $-\log$  of these measure-



ments was also plotted as ordinates (Fig. 4) to show the scattered points, especially those higher numbered glasses. The results show that the wave length at which a straight line touches most of the points on the graph is 430  $m\mu$ , which indicates that Beer's law is being followed. Then the mathematical relationship between the transmittance, T, for a solution containing a light absorbing substance of concentration, C, in a layer of unit thickness is  $I = I_0 \cdot 10^{-kc}$ , which may be written  $-\log I/I_0 = kc = -\log T$ .



A plot of the (C-T) data obtained at the various wave lengths produced certain curves—straight lines when a line was drawn through an average of the somewhat scattered points. Since these lines were intended for use as calibration curves it was necessary to test their suitability of application by measuring the transmittance of different concentrations of whiskey, caramel solutions, and beer in the spectrophotometer at the wave lengths previously mentioned and then applying the data to the curves. Per cent absorption was then read in a neutral wedge photometer by using a half-inch cell and a blue filter transmitting light at 460 mμ, and finally visual

readings were made in the Lovibond tintometer by means of the same half-inch cell that was used in the spectrophotometer, Table 2.

The only curve producing the desired relationship and that touches nearly all the points is the one made at the wave length of 430  $m\mu$  and for which distilled water was used as a reference solution. Therefore, this is the wave length at which colored solutions and colored glasses of this kind should be read when a 30  $m\mu$  exit slit is used, because if the negative

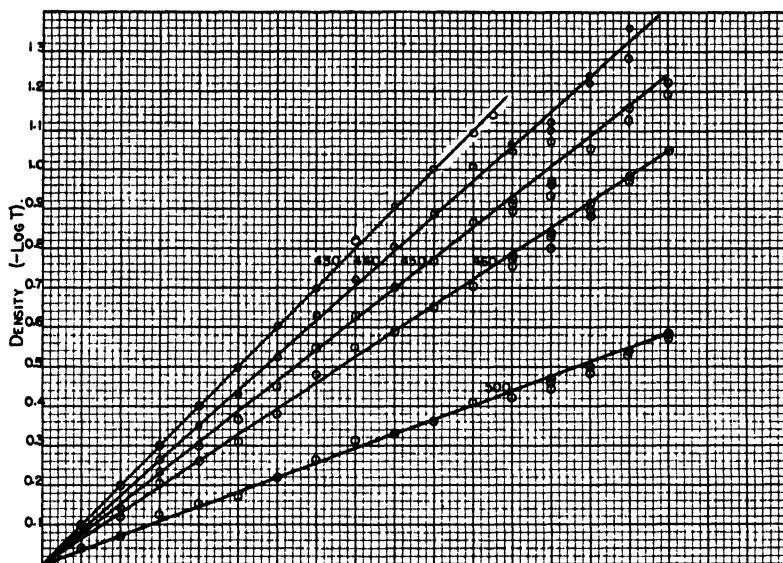


FIG. 4.—TRANSMITTANCE AS A FUNCTION OF LOVIBOND NUMBER AT WAVE LENGTHS INDICATED.

logarithm of the transmittance (density) is multiplied by 10 the resultant product will be equal to the Lovibond number shown in Table 1, which contains the transmittance, density, and the density multiplied by 10 at the wave length of 430  $m\mu$  of this laboratory's brown-colored glasses series No. 52 up to the number 12 glass.

An examination of Table 2 shows that a very close relationship exists between  $-(\text{Log } T) \times 10$  and the visual Lovibond reading as well as the neutral wedge photometer readings in terms of Lovibond number. It also shows that the variation between the numbers on the glasses and  $-(\text{Log } T) \times 10$  is not more than 0.2 in all except a few cases; however, the variation in most of the glasses is 0.1 or less.

### CONCLUSIONS

A method has been developed for correlating spectral transmittance of the brown-colored glasses, Series No. 52, recommended by the American Institute of Brewing Chemists, to the Lovibond number by using a spectrophotometer having a 30  $m\mu$  exit slit and by multiplying the negative

TABLE 2.—Comparison of transmittance ( $T$ ) at wave length of  $430\text{ m}\mu$  of whiskey, beer, ale, and wort and their respective densities multiplied by 10 with the visual readings of the same products in a Lovibond tintometer and neutral wedge photometer which had been calibrated to Lovibond number by using a blue filler transmitting light at  $460\text{ m}\mu$

$T$	$-(\log T)10$	VISUAL LOVIBOND READING	LOVIBOND NUMBER FROM NEUTRAL WEDGE PHOTOMETER
<i>Whiskey</i>			
0.615	2.1	2.0	2.0
0.560	2.5	2.5	2.4
0.415	3.8	4.0	3.7
0.428	3.7	3.5–4.0	3.5
0.313	5.05	5.0	5.0
0.292	5.35	5.5	5.5
0.235	6.3	6.5	6.5
0.168	7.75	7.5–8.0	8.0
0.134	8.7	9.0	9.1
0.105	9.8	10.0	10.0
<i>Beer</i>			
0.475	3.2	3.0	3.1
0.420	3.8	4.0	3.8
0.333	4.8	5.0–5.5	4.9
0.318	5.0	5.0–5.5	5.0
<i>Ale</i>			
0.462	3.35	3.5	3.3
0.512	2.9	3.0	2.8
<i>Wort</i>			
0.504	3.0	3.0	3.0
Some wort concentrated slowly with mild heat.			
0.188	7.3	7.0–7.5	7.4

logarithm of the transmittance (density) at the selected wave length ( $430\text{ m}\mu$ ) by 10. No calibration curve is necessary; however, by plotting the transmittance as a function of Lovibond number at the selected wave length on semi-logarithmic graph paper a curve (straight line) is obtained, the use of which would eliminate the calculations. It is also a simple and rapid procedure for the standardization of the glasses up to about the No. 12 glass. The transmittance above 12 is so low as to make the readings, or measurements, unreliable as evidenced by the scattered points in Fig. 4. The probable reasons why the points for the glasses from 12 up vary so much from the straight line in Fig. 4 are instrument errors at such low transmissions or the inability of the manufacturer of the glasses

to visually match them, owing to low transmission, or both. The same is true when the transmission is very high, as it is for glasses having a Lovibond number less than 0.5.

#### METHOD

Measure the transmittance of the glasses or solutions of a brown or amber color at the selected wave length, 430  $m\mu$ , using distilled water in a half-inch cell as a reference solution, and multiply the negative logarithm of the result by 10; or refer to the calibration curve for the corresponding Lovibond number. (In other words, the spectral densities ( $-\text{Log } T$ ) of the brown series of color glasses at the selected wave length is a direct linear function of the Lovibond number.)

NOTE: The results given in this paper are based on measurements made on a spectrophotometer equipped with a 30  $m\mu$  exit slit and doubly monochromated light.

### DETERMINATION OF ORGANICALLY COMBINED CALCIUM, BARIUM, AND STRONTIUM IN COAL-TAR COLOR PIGMENTS

By K. A. FREEMAN (U. S. Food and Drug Administration,  
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In this method the color pigment is decomposed by ammonium carbonate solution, the metal precipitating as the carbonate and the dye forming a soluble ammonium salt. Separation is accomplished by filtration, and the metal is determined by the usual procedures.

Ammonium carbonate was chosen because it does not decompose barium sulfate, which is present as substratum in many lakes. Even weak solutions of sodium and potassium carbonates react with barium sulfate to give barium carbonate. The method follows:

#### METHOD

*Reagent: Alcoholic ammonia—ammonium carbonate solution.*—Dissolve 20 grams of  $(\text{NH}_4)_2\text{CO}_3$  and 10 ml. of  $\text{NH}_4\text{OH}$  in 500 ml. of water. Dilute to 1 liter with 95% ethyl alcohol.

*A. Total Metals (calcium, barium, and strontium).*—Weigh 0.5000–0.7000 gram of the pigment into an 800 ml. beaker and add ca. 500 ml. of the carbonate reagent. Cover with a watch glass and heat on a steam bath until the color is in solution and a light colored precipitate appears on the bottom of the beaker. Decant the hot solution with suction through a Gooch crucible with an asbestos mat. Be careful not to pour any more of the precipitate into the crucible than necessary since rapid filtration is essential. If the dye crystallizes and clogs the filter, wash with a few ml. of hot carbonate reagent.

To the precipitate in the beaker add another 150–200 ml. of reagent and boil. Let settle and again decant into the crucible. Repeat this process until the washings are nearly colorless. Discard the filtrate.

To the residue in the beaker add ca. 100 ml. of acetic acid (1+10), being careful to wash the sides with the acid solution. Boil, and filter hot through the same Gooch crucible, connected to a clean receiver. (CAUTION: Add the hot acid solution in



small portions at first. Be sure suction is on before filtering.) Wash beaker with several small portions of distilled water and add them to the crucible.

Transfer the filtrate to a 400 ml. beaker, decolorize with a few drops of bromine water, and boil to expel excess bromine. Determine the metals in the usual way.

*B. Blank (soluble barium, calcium, or strontium salts present in the pigment).—*Weigh 5.000 grams of the color pigment into a 250 ml. volumetric flask. Add 175 ml. of water containing 5 ml. of glacial acetic acid. Stopper the flask and shake violently for ca. 5 minutes; dilute to volume and filter through a dry filter. Determine metals on a 200 ml. aliquot (equivalent to 4 grams of color pigment).

The difference between the percentage of metals found in A and in B is the percentage of metal, combined organically.

This method gives doubtful results for D&C Red No. 15, D&C Red No. 16, and D&C Red No. 31, because the proposed determination of the blank breaks down the organic combination of metal and dye in these pigments. For other pigments containing certified coal-tar colors it appears to give results corresponding to at least 98 per cent of the theoretical, based on the pure dye content of the pigment, as determined by titration with titanium trichloride.

Since most lakes of calcium, barium, and strontium are made by boiling the sodium salt of the dye with the appropriate metallic chloride this method has been found to be practical for checking the completeness of the conversion.

## BACTERIOLOGICAL AND PHYSICAL CHANGES OCCURRING IN FROZEN EGGS

### INFLUENCE OF DEFROSTING AND PROLONGED STORAGE ON BACTERIAL COUNT AND ON ODOR

By ROY SCHNEITER,\* M. T. BARTRAM, and H. A. LEPPER (U. S.  
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The bacterial changes occurring in frozen eggs during shipment and under conditions of commercial storage have received but scant attention within recent years, and a search of the literature reveals little information on this subject.

Stiles and Bates<sup>1</sup> packed, under laboratory conditions, various grades of eggs in approximately 50 ml. quantities, and stored them at 10°F. for a period of one year. Flasks of each product were removed at intervals for bacteriological examination. The study revealed little variation in the bacterial content of strictly fresh and commercially fresh frozen eggs during this period, while the change in checks, cracks, dirties, spots, and rots was even less pronounced, although a very gradual decline in the average total count of the various grades was apparent.

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<sup>1</sup> U. S. Dept. Agriculture Bull. 158 (1912).

Nielsen and Garnatz<sup>2</sup> obtained a sharp drop in the bacterial content of frozen eggs containing 14 per cent of salt when held at  $-18^{\circ}\text{C}$ . The greatest reduction occurred in 41 days, and thereafter (up to 208 days) little change was evident. Frozen eggs with 10 per cent of sugar failed to show a reduction in bacterial count until after 114 days, and there was a continued decrease through the 203rd day.

The experiments presented here were undertaken to show the bacterial and physical changes occurring in eggs of good and poor quality during the usual conditions of preparation, freezing, shipment, and storage, and during prolonged storage. The influence of improper methods of preparation and of insanitary plant practices on the quality of frozen eggs was ascertained. The effect on these products of thawing and refreezing was also studied to determine the influence of accidental defrosting.

### EXPERIMENTAL

*Preparation.*—All the experimental packs, consisting of 30 pound cans of eggs, were prepared during the latter part of May or early June in egg-breaking establishments in Kansas City under the usual commercial conditions, and they were frozen, shipped, and stored in the regular channels with commercial packs. They were divided into six groups, as shown in Table 1.

Samples of egg batter for bacteriological examination were removed from each can in the experimental packs immediately after the container was filled. These samples, well-stirred, were taken with aseptic precautions from the containers by means of sterile straight glass tubes ( $0.25'' \times 18''$ ). The odor and appearance of the product in each can were noted, after which all cans, except pack F, were immediately placed in a sharp freezer at from  $-10$  to  $-14^{\circ}\text{F}$ . The cans in pack F were slow frozen at  $0^{\circ}\text{F}$ . The cans were resampled approximately 60 hours after being placed in the freezer by drilling, with an electric drill and a one inch bit, three cores equidistant between the side and center of each can and one-third of the periphery apart. The chips from the top layer were discarded, and the others were collected in sterile jars. The sampling procedure used for these and subsequent samples was the same as that recommended by Schneider.<sup>3</sup> The frozen eggs were maintained at a temperature of from  $+5$  to  $-13^{\circ}\text{F}$ . for approximately three months and were then shipped to Washington, D. C. with a commercial car-lot shipment. Two special recording thermometers enclosed in regular 30 pound egg cans were included with the shipment, and records of re-icing were obtained. The temperatures recorded during transit varied from  $24^{\circ}$  to  $32^{\circ}\text{F}$ ., but never exceeded the latter figure. Immediately upon receipt the eggs were examined, samples

<sup>2</sup> First Food Technical Conference, 1940, pp. 289-294.

<sup>3</sup> *This Journal*, 22, 625 (1939).

were removed, and the cans were placed in commercial storage at from 0° to -5°F.

Approximately one month after shipment one can of each type of product (whites, yolks, or whole eggs) from each of packs A and B, two cans from each of packs C and E, and one can from each of D and F were

TABLE 1.—*Experimental packs*

PACKS	NO. OF CANS	TYPE OF PRODUCT	QUALITY OF SHELL EGGS	PLANT SANITATION
A	6	Whites	Selected eggs from good grade of current receipts consisting of fresh, sound, clean, uniform shell eggs.	Good
	6	Yolks		
	6	Whole		
B	6	Whites	Average run of good grade current receipts; included selected eggs as well as small, weak, and slightly cracked eggs.	Good
	6	Yolks		
	6	Whole		
C	1	Whole	Good egg batter % plus rots (%)	Good
	1	Whole	Good eggs 97 Rots 3	
	1	Whole	Good eggs 95 Rots 5	
	1	Whole	Good eggs 90 Rots 10	
	1	Whole	Good eggs 75 Rots 25	
	1	Whole	Good eggs 52 Rots 48	
D	2	Whole	Good eggs 0 Rots 100	Good
			Included checks, cracks, leakers, and washed dirties.	
E	1	Whole	Average run of good current receipts plus washed dirties	Fair
	1	Whole	Second-grade egg batter plus checks, cracks, and leakers.	Fair
F	2	Whole	Low-grade breaking stock and candling rejects.	Poor

removed from storage and placed on the roof of the storage plant in the sun, where they remained for six hours at temperatures ranging from 80° to 100°F. The cans were then removed to the interior of the building and left overnight (18 hours) at 64°F., at which time the contents were thawed, except for a mushy central core. The temperatures of the well-stirred product ranged from 37° to 46°F. The eggs were sampled and examined, and the cans were replaced in the freezer for refreezing, after which they were again examined.

After storage for one year in Washington the cans were sampled and examined. All of them, with the exception of one of each type of product from packs A and B, four cans from C, and one can from each of D and F, were removed from storage, thawed, and refrozen in the same manner as that outlined above except that the temperature in this instance did not exceed 95°F. during the thawing period. The temperatures of the product after exposure on the roof ranged from 36° to 44°F., and they had risen to 44°–60°F. by morning. Samples were removed from the liquid eggs and again after refreezing.

The cans noted previously, which were not removed for defrosting, were maintained in storage for a total period of six years, with examination after five and six years of storage.

*Bacteriological and Organoleptic Examination.*—All samples of egg batter and frozen egg drillings collected for bacteriological studies were examined according to the procedures outlined by Schneiter.<sup>3</sup> Total plate counts of viable microorganisms were determined by plating appropriate dilutions on dextrose agar with incubation at room temperature (25°–32°C.) and at 37°C. for 72 hours. The incidence of the coliform group of bacteria was determined by lactose broth presumptive test with partial confirmation on Levine's eosin-methylene blue agar. Hemolytic staphylococci and streptococci and anaerobic microorganisms were determined on veal blood agar and alkaline cooked-meat medium, respectively; however, the results were of no apparent significance and are not included in this study.

The results of the bacteriological and physical examination are given in Table 2. The individual cans of packs A and B are not listed, but the results are summarized. The counts obtained at 37°C. are also not given, but in general they were consistently lower than those obtained at the lower incubation temperatures.

The results of the first analysis made on the unfrozen batter are typical for the various types of eggs used. All the eggs in pack A were normal, and the bacteriological counts ranged from less than 10,000 to 30,000 per gram, with members of the coliform group never present in dilutions greater than 1:1000. In pack B the counts were somewhat higher, but not in excess of 300,000, except in the case of the whole eggs, where counts up to 2,000,000 per gram were obtained. A portion of the batter used in filling the six cans in this lot had stood in the churn over the lunch period (30 minutes) under hot summer conditions, and the same conditions prevailed in the first can of pack F. It presented a noticeably strong odor although the bacterial content is not high. The eggs in pack C were definitely putrid, and the counts correspondingly high, while the results on packs D and E indicate normal eggs with the variable counts encountered when dirty eggs or cracked leaky eggs are used.

*Influence of Freezing and Shipment.*—The results of the second analysis show decreases in bacterial count typical of those obtained on freezing

TABLE 2.—*Influence of shipment and defrosting on odor and bacterial content of frozen eggs under short-time storage*

PACK	TYPE	FIRST ANALYSIS BEFORE FREEZING						SECOND ANALYSIS 2½ DAYS AFTER FREEZING						THIRD ANALYSIS AFTER SHIPPING 2½-3 MONTHS AFTER PACKING						FOURTH ANALYSIS BEFORE DEFROSTING						FIFTH ANALYSIS DEFROSTED						SIXTH ANALYSIS REFROZEN					
		ODOR		COUNT/GM.†	COLI-FORM* FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM	ODOR		COUNT/GM.	COLI-FORM				
A	Whites	(1) Normal	17,000	+2	7,000	+1	(1) Normal	(1) Normal	3,950	—(1 gm.)	200	—	(5) Normal	(5) Normal	1,000	—	(5) Normal	1,000	—	(5) Normal	152	—	(5) Normal	152	—	(5) Normal	152	—	(5) Normal	152	—	(5) Normal	152				
	Yolks	(1) Normal	28,000	+1	19,000	+1	(1) Normal	(1) Normal	7,300	+1	9,000	+1	(5) Normal	(5) Normal	13,200	—	(5) Normal	13,200	—	(5) Normal	5,740	+1	(5) Normal	5,740	+1	(5) Normal	5,740	+1	(5) Normal	5,740	+1	(5) Normal	5,740				
	Whole	(1) Normal	10,000	+1	1,500	—(0.1 gm.)	(1) Normal	(1) Normal	1,600	—	700	+1	(5) Normal	(5) Normal	2,860	—	(5) Normal	2,860	—	(5) Normal	1,460	+1	(5) Normal	1,460	+1	(5) Normal	1,460	+1	(5) Normal	1,460	+1	(5) Normal	1,460				
B	Whites	(1) Normal	150,000	+4	77,000	+3	(1) Normal	(1) Normal	64,300	+2	47,300	+1	(5) Normal	(5) Normal	122,000	+1	(5) Normal	122,000	+1	(5) Normal	115,000	+1	(5) Normal	115,000	+1	(5) Normal	115,000	+1	(5) Normal	115,000	+1	(5) Normal	115,000				
	Yolks	(1) Normal	128,000	+4	111,500	+4	(1) Normal	(1) Normal	215,000	+3	1,329,000	+3	(5) Normal	(5) Normal	2,082,000	+3	(5) Normal	2,082,000	+3	(5) Normal	19,999,000	+3	(5) Normal	19,999,000	+3	(5) Normal	19,999,000	+3	(5) Normal	19,999,000	+3	(5) Normal	19,999,000				
	Whole	(2) Normal	1,800,000	+4	1,730,000	+5	(1) Strong	(1) Strong	3,200,000	+5	32,210,000	+6	(5) Strong	(5) Strong	18,400,000	+3	(5) Putrid	18,400,000	+3	(5) Putrid	22,520,000	+5	(5) Putrid	22,520,000	+5	(5) Putrid	22,520,000	+5	(5) Putrid	22,520,000	+5	(5) Putrid	22,520,000				
C	rots 3	Stale	16,000,000	+6	8,600,000	+6	Stale	Stale	42,000,000	+7	256,000,000	+7	(4) Putrid	(4) Putrid	220,000,000	+6	(4) Putrid	220,000,000	+6	(4) Putrid	590,000,000	+6	(4) Putrid	590,000,000	+6	(4) Putrid	590,000,000	+6	(4) Putrid	590,000,000	+6	(4) Putrid	590,000,000				
	rots 5	Putrid	29,000,000	+6	8,900,000	+6	Putrid	Putrid	60,000,000	+7	128,000,000	+7	(4) Putrid	(4) Putrid	340,000,000	+7	(4) Putrid	340,000,000	+7	(4) Putrid	490,000,000	+7	(4) Putrid	490,000,000	+7	(4) Putrid	490,000,000	+7	(4) Putrid	490,000,000	+7	(4) Putrid	490,000,000				
	rots 10	Putrid	22,000,000	+6	8,000,000	+6	Putrid	Putrid	65,000,000	+7	158,000,000	+7	(4) Putrid	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000	+7	(4) Putrid	151,000,000				
D	rots 25	Putrid	150,000,000	+6	8,000,000	+6	Putrid	Putrid	23,000,000	+7	80,000,000	+7	(4) Putrid	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000	+7	(4) Putrid	80,000,000				
	rots 48	Putrid	350,000,000	+6	53,000,000	+7	Putrid	Putrid	120,000,000	+8	91,000,000	+7	(4) Putrid	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000	+7	(4) Putrid	91,000,000				
	sour 100	Sour	270,000,000	+6	240,000,000	+7	Sour	Sour	52,000,000	+8	29,600,000	+4	(4) Putrid	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000	+4	(4) Putrid	29,600,000				
E	Whole	Normal	10,000	+2	62,000	+2	Normal	Normal	10,000,000	+4	60,000	—	(4) Putrid	(4) Putrid	47,000,000	+6	(4) Putrid	47,000,000	+6	(4) Putrid	1,100,000	—	(4) Putrid	1,100,000	—	(4) Putrid	1,100,000	—	(4) Putrid	1,100,000	—	(4) Putrid	1,100,000				
	Whole	Normal	4,900,000	+6	1,600,000	+6	Normal	Normal	5,300,000	+6	7,000	—	(4) Normal	(4) Normal	8,000,000	+5	(4) Normal	8,000,000	+5	(4) Normal	200,000	—	(4) Normal	200,000	—	(4) Normal	200,000	—	(4) Normal	200,000	—	(4) Normal	200,000				
	Whole	Strong	18,000,000	+6	1,900,000	+5	Strong	Strong	21,000,000	+6	35,000,000	+6	(4) Putrid	(4) Putrid	13,000,000	+6	(4) Putrid	13,000,000	+6	(4) Putrid	52,000,000	+7	(4) Putrid	52,000,000	+7	(4) Putrid	52,000,000	+7	(4) Putrid	52,000,000	+7	(4) Putrid	52,000,000				
F	Whole	(2) Strong	680,000	+4	470,000	+4	Strong	Strong	5,100,000	+5	10,000,000	+5	(4) Stale	(4) Stale	16,000,000	+4	(4) Stale	16,000,000	+4	(4) Stale	83,000,000	+8	(4) Stale	83,000,000	+8	(4) Stale	83,000,000	+8	(4) Stale	83,000,000	+8	(4) Stale	83,000,000				
	Whole	Normal	2,800,000	+5	270,000	+5	Normal	Normal	8,600,000	+5	2,000,000	+4	(4) Strong	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000				
	Whole	Normal	2,800,000	+5	270,000	+5	Normal	Normal	8,600,000	+5	2,000,000	+4	(4) Strong	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000	+4	(4) Strong	2,000,000				

\* Reciprocal of dilution; i.e., 2 = positive in 1:100 dilution, etc.; — = negative in 1:10 dilution.

† All plates incubated at room temperature (25°-32°C.).

(1) Average of 6 cans.

(2) Stood 30 minutes before filling (summer temperature).

(3) One can from each group defrosted and refrozen, after third analysis.

(4) Can defrosted and refrozen, after third analysis.

(5) Average of 6 cans.

the egg batter. The odor of the eggs in the various packs remained unchanged except for the whole eggs of pack B, which had become noticeably strong. The bacterial counts on these had increased slightly over those of the batter.

The results of the third analysis, made after a storage period of approximately three months and after shipment to Washington, D. C., indicate that except for a slightly decreased count no significant change had occurred in the good eggs of pack A. However, in the remaining packs the bacterial counts had undergone slight to marked increases, although in no instance could any change in the odor of the product be noted. This condition indicates that the increase in bacteria occurred during the shipping and that although the temperatures, as shown by the recording thermometers, never exceeded 32°F., they were sufficiently high for bacterial increases to occur in those packs that contained abnormal eggs and had high original bacterial content. This should be contrasted with the eggs of good quality.

*Influence of Defrosting.*—The 12 cans defrosted after the third analysis were sampled while in the thawed state and again after refreezing. These results are given in Table 3. In general, all of the eggs so treated showed slight increases in bacterial count while in the liquid state, with the most marked increase occurring in packs A and B. The number of organisms observed in the mixed defrosted product in no instance exceeded 24 times those in the product prior to thawing. This is in sharp contrast to the results obtained by Brownlee and James,<sup>4</sup> who observed average increases of 180, 360, and 660 per cent in samples taken from the center, midway, and outside positions of cans during a 24-hour defrosting period in water at 11.5°C. On refreezing, the total counts, except for the can of whole eggs in pack B, returned to the levels observed on the third analysis. The odors of the thawed eggs in pack A and the whites and yolks of pack B were normal, while the defrosted can of whole eggs in pack B was definitely putrid, as were the eggs in pack D and in one can of pack E.

The results of the examination after the eggs had been in storage one year are recorded under the fourth analysis (Table 2). Continued decreases in the bacterial counts in pack A were obtained, particularly in the whites and whole eggs, and all were normal in odor and appearance. The cans that had been defrosted one year previous to this analysis were entirely similar in all respects to the ones not so treated. Pack B showed some decrease in the average counts on the whites, while the yolks and whole eggs had undergone increases in bacterial content. All of the eggs in this pack remained normal except that the whole eggs had the same "strong" odor noted on freezing and the previously defrosted can was putrid.

The eggs in packs C, D, E, and F showed definite increases in bacterial counts with the exception that one of the defrosted cans in each of D and

<sup>4</sup> Proc. Seventh World's Poultry Congress, 1939, pp. 488-492.

TABLE 3.—Results of preliminary defrosting on odor and bacterial content of frozen eggs

PAGE	TYPE	BEFORE DEFROSTING			THAWED			REFROZEN		
		ODOR	COUNT*/GRAM	COLIFORM†	ODOR	COUNT/GRAM	COLIFORM	ODOR	COUNT/GRAM	COLIFORM
A	Whites	Normal	1,900	Neg.	Normal	4,900	Neg.	Normal	6,700	Neg.
	Yolks	Normal	7,000	Neg.	Normal	110,000	0.5	Normal	27,000	0.5
	Whole	Normal	2,000	Neg.	Normal	8,000	2	Normal	4,000	Neg.
B	Whites	Normal	6,000	2	Normal	110,000	2	Normal	76,000	2
	Yolks	Normal	120,000	2	Normal	2,900,000	2	Normal	250,000	2
	Whole	Normal	4,100,000	6	Sl. putrid	27,000,000	6	Putrid	13,000,000	5
C	3% rots	Stale	42,000,000	7	Putrid	18,000,000	7	Putrid	14,000,000	7
	5% rots	Putrid	60,000,000	7	Putrid	20,000,000	5	Putrid	17,000,000	6
D	Whole	Normal	5,300,000	6	Stale	7,000,000	6	Putrid	6,800,000	6
E	Whole	Normal	560,000	5	Normal	310,000	6	Normal	100,000	4
	Whole	Strong	21,000,000	6	Sl. putrid	9,600,000	5	Strong	7,700,000	6
F	Whole	Strong	5,100,000	5	Stale	1,500,000	4	Putrid	2,800,000	4

\* Incubated at room temperature (25°-30°C.).

† Reciprocal of dilution.

E gave a much lower count. No changes in odor from the last analysis were noted in the cans not previously defrosted.

Immediately following the fourth analysis all of the cans, with the exception of 12 cans that were to be continued in storage, were thawed and examined, then refrozen and again examined. These results are recorded under the fifth and sixth analyses, respectively. Increased counts of 5-, 1.5-, and 4-fold were observed in the whites, yolks, and whole eggs, respectively, of pack A while in liquid state, but the count returned to the prethawed level, or below, on refreezing. All of the eggs in this pack remained normal, although this was the second defrosting for one can in each of the product types. In pack B the average counts of the yolks underwent a 10-fold increase during thawing and refreezing, and the contents were observed to be either quite strong or slightly putrid. The egg whites in this pack remained normal, and the bacterial counts were not significantly changed by the defrosting.

All the eggs in the remaining packs showed increases in bacterial count, and all were abnormal in odor. In all the analyses it will be observed that the coliform content roughly parallels the total bacterial count and therefore serves as a valuable adjunct in measuring the quality of the product.

*Effect of Prolonged Storage on Frozen Eggs.*—The 12 cans of eggs from the previous experiment that were not subjected to defrosting after one year in storage, consisting of one can from each type of product in packs A and B, four cans from pack C, and one can from each of packs D and F, were continued in storage for a total period of six years, with examination at the end of five years and again after six years in storage. At this latter date the cans themselves were quite deteriorated, and it seemed advisable to terminate the experiment.

The results of the organoleptic and bacteriological examination of these samples are given in Table 4. The first three analyses are the same as those previously recorded in Table 2 and will not be discussed again.

The physical condition of the eggs after storage for five and six years is quite interesting. Except for the presence of large ice crystals and a leathery texture, which did not affect the edibility of the product after defrosting, all the eggs in packs A, B, D, and F were quite normal in appearance and in odor. The whole eggs in pack B and the eggs in pack C (containing rots) presented a disintegrated appearance and a strong or putrid odor. The odor was much less intense than was the case at the end of one year's storage and became progressively less pronounced after six years' storage.

The bacterial count of the eggs of good quality (pack A) underwent but little change in the five-year period between the fourth and sixth examinations. The egg whites in packs A and B were practically free from bacteria, containing less than 10 per gram, which, in the case of pack B, was a definite decrease. This was undoubtedly due to the presence of bacteri-



TABLE 4.—*Effect of prolonged storage on odor and bacterial content of frozen eggs*

PACK	TYPE	IMMEDIATELY AFTER FREEZING			THREE MONTHS' STORAGE			ONE YEAR'S STORAGE			FIVE YEARS' STORAGE			SIX YEARS' STORAGE		
		ODOR	COUNT/GRAM†	COLI-FORM*	ODOR	COUNT/GRAM	COLI-FORM	ODOR	COUNT/GRAM	COLI-FORM	ODOR	COUNT/GRAM	COLI-FORM	ODOR	COUNT/GRAM	COLI-FORM
A	Whites	Normal	7,000	+2	Normal	5,700	—	Normal	<10	—	Normal	<10	—	Normal	<10	—
	Yolks	Normal	17,000	+2	Normal	5,000	+2	Normal	7,900	+1	Normal	4,000	+2	Normal	2,700	—
	Whole	Normal	3,000	+2	Normal	3,000	—	Normal	600	+1	Normal	200	—	Normal	400	—
B	Whites	Normal	42,000	+3	Normal	58,000	+2	Normal	186,000	+2	Normal	<1,000	—	Normal	<10	—
	Yolks	Normal	100,000	+5	Normal	270,000	+3	Normal	84,000	—	Normal	33,000	+2	Normal	6,500	+4
	Whole	Strong	1,100,000	+5	Strong	6,200,000	+7	Strong	1,700,000	+5	Strong	1,400,000	+5	Strong	106,000	+4
C	10% rots	Putrid	8,000,000	+6	Putrid	65,000,000	+7	Putrid	158,000,000	+7	Putrid	2,500,000	+4	Putrid	660,000	+5
	25% rots	Putrid	8,000,000	+6	Putrid	23,000,000	+7	Putrid	80,000,000	+7	Putrid	14,000,000	+7	Putrid	3,300,000	+6
	48% rots	Putrid	53,000,000	+7	Putrid	120,000,000	+8	Putrid	151,000,000	+7	Putrid	14,000,000	+7	Putrid	6,200,000	+6
	100% sour	Sour	240,000,000	+7	Sour	52,000,000	+8	Putrid	91,000,000	+7	Putrid	3,000,000	+6	Putrid	700,000	+5
D	Cheeks, etc.	Normal	62,000	+2	Strong	10,000,000	+4	Strong	29,600,000	+4	Normal	220,000	+3	Normal	<1,000	—
F	Rejects	Normal	270,000	+5	Normal	8,600,000	+5	Strong	2,100,000	+4	Normal	130,000	+2	Normal	11,000	—

† Plates incubated at room temperature (25°–30°C.).

\* Reciprocal of dilution; i.e., +3 = positive in 1:1000 dilution; — = negative in 1:10 dilution.

cidal lysozyme in the egg white, and it may also account for the low counts encountered in the whole eggs as compared to the yolk containing no egg white. The whole eggs in pack B yielded considerably fewer organisms after six years' than after one year's storage; however, the count was significantly higher than that on the other eggs in either pack A or B and still reflected the abuse to which those eggs were subjected when they were held in the churn prior to being placed in the cans.

The bacterial counts of the eggs containing varying percentages of rots likewise underwent a decrease during the five-year period, but they were still of such magnitude that the eggs could probably not be considered an acceptable product. The counts in packs D and F had decreased to a level similar to those of the eggs of good quality of pack A, and except for the high count and strong odor encountered immediately after shipment and after one year's storage, these eggs were never an unsatisfactory product.

#### SUMMARY AND CONCLUSIONS

Studies were undertaken in order to ascertain the bacterial and physical changes occurring in eggs of good and poor quality during the usual conditions of preparation, freezing, commercial storage, and prolonged storage, and the influence on the quality of frozen eggs of insanitary plant practices and improper methods of preparation. Commercial 30 pound cans of frozen eggs were prepared from eggs of good quality, selected from average fresh shell stock, and from several types of eggs of poor quality, e.g., checks, cracks, leakers, washed dirties, and those containing varying percentages of rots. These experimental packs were subjected to the usual conditions of shipment and storage and to intentional defrosting.

Bacterial and physical changes observed lead to the following conclusions:

- (1) Frozen eggs of good quality are able to withstand at least two complete thawings and refreezings without significant change in bacterial content or without acquiring abnormal appearance or odor.

- (2) Eggs of poor quality, including cracks, leakers, and dirty eggs, usually have high bacterial counts. This condition leads to progressive decomposition of the product unless it is rapidly frozen and maintained in the frozen condition. Insanitary plant practices and improper methods of preparation of the egg batter are conducive to rapid decomposition, especially when freezing is delayed or prolonged.

- (3) Prolonged storage of frozen eggs over a period of six years resulted in a considerable reduction of the bacterial content, but the total count still served as a reliable index of the original quality of the product. The counts after six years' storage ranged from 300 per gram in whole eggs of good quality to over 6,000,000 in second grade eggs containing 48 per cent rots. The physical condition of the frozen egg products did not change

during prolonged storage, except for the formation of ice crystals and small leather-like lumps of separated egg solids in the whole eggs and egg yolks. The odor remained unchanged from that recorded immediately after freezing although that of the putrid eggs became less intense.

(4) Three per cent of rots, which was the lowest amount used in these studies, could be readily detected by an experienced egg examiner.

(5) The total plate count and the coliform index are roughly parallel, and each may serve as a reliable index of the original quality of the product.

(6) There is a rapid reduction in the numbers of viable microorganisms in frozen egg whites, which may be attributed to the presence of the bactericidal lysozyme.

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### SEMIMICRO METHOD FOR DETERMINATION OF SULFUR IN ORGANIC SUBSTANCES

By J. H. JONES (Cosmetic Division, U. S. Food and Drug  
Administration, Washington, D. C.)

The oxidation of organic sulfur compounds by a modification of Kahane's method, followed by titration of the sulfate produced with barium chloride, using tetrahydroxyquinone as an indicator, has been found to be a simple, rapid method for the determination of semimicro amounts of organic sulfur.

Kahane and Kahane<sup>1</sup> used a mixture of nitric, perchloric, and iodic acids to oxidize organic sulfur compounds. They determined the sulfate formed by precipitation as barium sulfate, after reducing the iodate remaining in the digestion mixture to iodide. Since either iodate or iodide interferes in the proposed titration of sulfate, iodic acid was not used in the proposed method. However, a number of organic sulfur compounds can be quantitatively oxidized by a mixture of nitric and perchloric acids alone. Other compounds that do not give quantitative results with the nitric-perchloric acid mixture are quantitatively oxidized if treated with aqua regia before the digestion with the nitric-perchloric acid mixture. The proposed procedure, therefore, specifies the use of aqua regia.

Many organic compounds react vigorously if digested with a mixture of concentrated nitric and perchloric acids. It is necessary, therefore, to begin the digestion in dilute solution and to concentrate the oxidation mixture gradually by boiling. With this procedure, the oxidation proceeds smoothly and the mechanical losses due to rapid reaction, shown by Wolesensky<sup>2</sup> to be the chief cause of low results in the determination of sulfur in rubber by Kahane's method, are avoided.

Nitrate ion in considerable concentration interferes with the proposed

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<sup>1</sup> *Bull. Soc. Chim.*, (5), 1, 280 (1934).

<sup>2</sup> *Ind. Eng. Chem.*, 20, 1234 (1928).

titration, but it is possible to carry out the titration accurately in the presence of perchlorate ion. Interference of the nitrate ion is prevented by volatilization of the excess nitric acid from hot perchloric acid solution. Perchloric acid forms a constant-boiling mixture with water, which boils at 203°C. At this temperature nitric acid is almost completely eliminated from mixtures of nitric and perchloric acids,<sup>3</sup> whereas sulfuric acid, if present in low concentration, is not appreciably volatilized.

The titration of sulfate with barium chloride and tetrahydroxyquinone indicator gives a sharp, readily observed end point when the titration is performed in the presence of silver chloride, as proposed by Mahoney and Mitchell.<sup>4</sup> However, when perchloric acid is neutralized with ammonium hydroxide and the excess ammonia is removed by boiling, the titration blank varies considerably. The use of boric acid to buffer the titration solution was found to be effective in producing a more constant blank without interfering with the titration.

#### METHOD

##### REAGENTS

*Nitric-perchloric acid mixture.*—Mix 2 volumes of 70%  $\text{HNO}_3$  and 1 volume of 72%  $\text{HClO}_4$ .

*Phenolphthalein indicator.*—Dissolve 1 gram of phenolphthalein in 100 ml. of ethyl alcohol.

*Dilute ammonium hydroxide.*—Approximately 0.05 *N* solution.

*Dilute perchloric acid.*—Approximately 0.05 *N* solution.

*Saturated boric acid solution.*—Approximately 5 grams of  $\text{H}_3\text{BO}_3$  per 100 ml. of  $\text{H}_2\text{O}$ .

*Ethyl alcohol.*—95%.

*Tetrahydroxyquinone indicator.*—"THQ Prepared Sulfate Indicator" is supplied by W. H. and L. D. Betz, Philadelphia, Pa. A measuring dipper, capacity ca. 0.15 gram, is furnished with the indicator.

*Ammonium chloride solution.*—Dissolve 10 grams of  $\text{NH}_4\text{Cl}$  in 100 ml. of water.

*Silver nitrate solution.*—Dissolve 10 grams of  $\text{AgNO}_3$  in 100 ml. of water.

*Barium chloride solution.*—0.02 *M*. Dissolve 4.886 grams of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  in 1 liter of water. Standardize by precipitation as  $\text{BaSO}_4$ .

##### PROCEDURE

**CAUTION:** Some substances react with explosive violence when digested with perchloric acid. Although the proposed procedure has been found to be safe with practically all types of compounds, a trial digestion of a small sample should be conducted before an attempt is made to analyze a substance not previously investigated.

Weigh out a sample containing 2–6 mg. of sulfur and transfer it to the bottom of a 30 ml. Kjeldahl flask. (An aliquot of an aqueous solution may be used as the sample.) Add 3–5 ml. of water, 1 ml. of  $\text{HCl}$  (1+1), 3 ml. of the  $\text{HNO}_3$ - $\text{HClO}_4$  mixture, and 2 or 3 glass beads. Place the flask on the digestion stand and boil gently until all the nitric acid is removed and the boiling perchloric acid condenses about two thirds of the way up the neck of the flask. The solution is usually clear at this

<sup>3</sup> "Perchloric Acid," Vol. 1, 4th ed., p. 10. Compiled by G. F. Smith. The G. Frederick Smith Company, Columbus, Ohio (1940).

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 97 (1942).

point; if not, continue to heat until the solution clears and then heat at the boiling point of  $\text{HClO}_4$  for 5–10 minutes. Remove the flask from the digestion stand, cool, dilute the digestion mixture to 8–10 ml. with water and transfer to a 200 ml. Erlenmeyer flask. Rinse the digestion flask with three 5 ml. portions of water and add them to the main solution. Add 3 drops of phenolphthalein indicator and neutralize the solution with concentrated  $\text{NH}_4\text{OH}$ . Place the flask on a hot plate and boil for 2–3 minutes after the pink color is discharged. (The solution should be colorless at this point; a yellow or brown color indicates that the digestion has not been con-

TABLE 1.—*Determination of known quantities of sulfate*

SUBSTANCE	AMOUNT OF 0.02 M SOLUTION ADDED	0.02 M $\text{BaCl}_2$ USED	APPARENT BLANK
	ml.	ml.	ml.
$\text{K}_2\text{SO}_4$	5.00*	5.10	0.10
	5.00*	5.08	0.08
	5.00	5.05	0.05
	5.00	5.10	0.10
	5.00*	5.10	0.10
	5.00*	5.05	0.05
	5.00	5.07	0.07
	5.00	5.12	0.12
$\text{NH}_4\text{SO}_4\text{H}$	3.00	3.12	0.12
	6.00	6.13	0.13
	7.00	7.05	0.05
	8.00	8.07	0.07
	9.00	9.13	0.13
Av.			0.09

\*  $\text{K}_2\text{SO}_4$  added after digestion was completed.

tinued long enough.) Adjust the volume of solution to  $25 \pm 5$  ml. by further boiling, or addition of water. Cool the contents of the flask to room temperature and add 0.05 N  $\text{NH}_4\text{OH}$  until the solution is a faint pink color. Add 2 ml. of the saturated  $\text{H}_3\text{BO}_3$  solution to discharge the pink color. If the color does not disappear, add 0.05 N  $\text{HClO}_4$  until the solution is colorless. Add 25 ml. of alcohol, 1 ml. of 10%  $\text{NH}_4\text{Cl}$  solution, and 1 dipper of tetrahydroxyquinone indicator, and shake until the indicator is dissolved. Add the standardized  $\text{BaCl}_2$  slowly until 1–2 ml. from the expected end point. Add 3 drops of 10%  $\text{AgNO}_3$  and continue the titration, adding the  $\text{BaCl}_2$  dropwise. Agitate the solution thoroughly throughout the titration. The end point is the appearance of a rose-pink color throughout the solution.

The change in color from yellow to pink at the end point is usually very sharp and easily detected after a few practice titrations. It is preferable to carry out the titration in natural light but the end point can be observed in strong artificial light.

#### BLANK DETERMINATION

Carry out a digestion with the same quantities of reagents as in the analysis but omit the sample. Wash the digestion mixture into a flask containing a known amount of sulfate and continue as directed in the regular determination. The ml. of  $\text{BaCl}_2$  required in excess of the calculated quantity is the combined reagent and titration blank. 1 ml. of 0.02 M  $\text{BaCl}_2$  = 0.642 mg. of S.

TABLE 2.—*Determination of sulfur in organic compounds*

SUBSTANCE	SAMPLE SIZE	SULFUR	
		FOUND	CALCULATED
	<i>mg.</i>	<i>per cent</i>	<i>per cent</i>
m-Nitrobenzene			
sulfonic acid	29.1	10.8	10.9
benzidine salt	52.1	10.9	
	37.8	10.9	
	35.1	10.9	
Sulfanilic acid*	25.0†	18.3	18.5
	25.0†	18.4	
	25.0†	18.4	
	25.0†	18.3	
Sulfanilamide*	30.0†	18.6	18.6
	30.0†	18.4	
2-Naphthylamine-	29.7	14.3	14.4
1-sulfonic acid	28.7	14.5	
p-Sulfo-phenyl-	30.0†	12.4	12.6
3-methyl-5-pyrazalone	30.0†	12.5	
Sulfathiazole*	22.5	25.1	25.1
	18.3	24.8	
Thiourea	10.0†	42.3	42.1
	10.0†	42.1	
Benzyl thiouronium	30.0†	15.6	15.8
chloride	30.0†	15.6	
	30.0†	15.6	
p-Nitro benzyl	16.3	19.1	19.1
disulfide	21.8	19.1	
p-Nitro benzyl sulfide*	38.1	10.4	10.5
	46.0	10.3	
	27.8	10.5	
	29.0	10.6	

\* Quantitatively oxidised only if treated with aqua regia.

† Sample consisted of a 5 ml. aliquot of a master solution.

## DISCUSSION

When the proposed method of analysis is used the sample may be either a weighed amount of the substance or an aliquot of an aqueous solution. Many organic sulfur compounds are soluble in water so that the use of aliquots is a convenient method of obtaining micro or semimicro samples accurately with an ordinary balance.

The time required for the digestion of a sample is about 30 minutes and for the subsequent operations about 15 minutes. However, if a number of analyses are made in series the average time required for each analysis can be reduced to 15-20 minutes.

The proposed method was developed primarily for the analysis of sulfonic acid derivatives of coal-tar colors, but it appears to be applicable to most sulfur compounds that are not volatilized from the digestion mixture before they are oxidized. Sulfonal is the only non-volatile compound investigated that was not quantitatively oxidized by treatment with aqua regia followed by digestion with the nitric-perchloric acid mixture.

Results of the analysis of known amounts of sulfate are shown in Table 1. In several of these determinations the digestion was continued for 20-30 minutes at the boiling point of perchloric acid without lowering of the recovery. These results show that if the oxidation is quantitative the method should be accurate to within  $\pm 0.05$  ml., of 0.02 *M* BaCl<sub>2</sub>.

Results of the analysis of representative compounds are shown in Table 2. If all the compounds are assumed to be pure, the average recovery of sulfur is 99.5 per cent.

Adaptation of the method to the analysis of micro samples is under study.

#### SUMMARY

A simple, rapid method for the determination of semimicro quantities of organic sulfur has been presented. The combined sulfur in non-volatile organic compounds is oxidized to inorganic sulfate by a mixture of nitric, hydrochloric, and perchloric acids and titrated with barium chloride. Tetrahydroxyquinone is used as an indicator.

Typical results are given.

#### DETERMINATION OF CAROTENE OXIDASE IN LEGUME SEEDS

By RAYMOND REISER and G. S. FRAPS (Agricultural Experiment Station, College Station, Texas)

In 1939 Sumner and Dounce<sup>1</sup> verified the observation of Haas and Bohn<sup>2</sup> that there is an enzyme in beans which, when mixed with carotene in oil solution, bleaches the carotene. Since the enzyme forms peroxides of unsaturated fatty acids, these authors proposed a method of determination based on the development and determination of the peroxides in cotton seed oil. The following year Sumner and Sumner<sup>3</sup> proposed a different method based on the length of time required for the complete

<sup>1</sup> *Enzymologia*, 7, 130 (1939).

<sup>2</sup> *Chem. Abstracts*, 28, 4137 (1936).

<sup>3</sup> *J. Biol. Chem.*, 134, 831 (1940).

bleaching of a carotene solution in acetone and alcohol to which the extract of beans and a buffer solution were added. They reported the optimum temperature to be 40°–45°C., the optimum pH 6.5, and the activity of the enzyme dependent upon the presence of unsaturated fatty acids, which it first oxidizes to peroxides, which in turn transfer the oxygen to the carotene. The rate of response was found to depend not only upon the degree of unsaturation of the fatty acids but also upon their amount, since large quantities of oil dissolve the carotene and protect it from the action of the enzyme.

Strain<sup>4</sup> has recently determined that the oxidase in legumes responsible for the bleaching of carotene and other pigments does not act directly on



them but only on compounds containing the  $-\text{C}=\text{C}-(\text{CH}_2)_7-\text{C}(\text{O})$  group in the cis configuration. The unstable oxides that result oxidize the pigments. Tauber<sup>5</sup> has also found that the so-called "Carotene oxidase" is an "unsaturated fat oxidase" and does not act directly on pigments.

At the time of the appearance of these papers an investigation was in progress at this laboratory to determine chemically the carotene-destroying power of meat and bone scraps and tankage. These feeds had been found to produce vitamin A deficiency symptoms when fed to chickens receiving what was considered to be adequate quantities of that vitamin. Considering the possibility that the presence of legumes might also increase the vitamin A requirement of animals because of the presence of the carotene oxidase, a practical method for determining the presence and amount of carotene oxidase in animal feeds was undertaken.

#### EXPERIMENTAL

In the first experiments, 1 gram of bean meal was stirred mechanically for 5 minutes with 50 ml. of water and 1 ml. of a 0.01 per cent solution of carotene in Wesson oil, and the residual carotene was determined. The large volume and the presence of the bean meal made extraction of the residual carotene difficult and repetition of the determination gave widely different results. The procedure was then modified so that an aqueous extract of the bean meal was stirred with the carotene-oil solution. Although this improved the extraction, results were still not reproducible.

At this time the paper by Sumner & Sumner<sup>3</sup> appeared, and the procedure was modified to conform to the facts presented by them. A solution of carotene and Wesson oil in acetone was used instead of a solution of carotene in oil. It was found that the alcohol-acetone mixture used by Sumner & Sumner<sup>3</sup> would not dissolve the desired quantities of carotene. The 2.5 per cent extract of bean meal used by those authors was found to

<sup>4</sup> *J. Am. Chem. Soc.*, 63, 3542 (1941).

<sup>5</sup> *Ibid.*, 62, 2251 (1940).



be too strong and, for preliminary work, a 1 per cent extract was used instead. Their buffer solution was adopted.

After several attempts to control aeration by stirring the reaction mixture with a mechanical stirrer, this procedure was given up and, instead, a small volume of mixture with a large and reasonably constant surface was used. It was found that 5 ml. of enzyme-buffer mixture and 1 ml. of substrate would little more than cover the bottom of a 200 ml. Erlenmeyer flask. This meets the requirements of a large and reasonably constant surface.

It was then necessary to determine the best proportions of oil and carotene in acetone. A series of experiments showed that a 0.06 per cent solu-

TABLE 1.—*Effect of 1 mg. of Laredo soy bean on different quantity of carotene*

CAROTENE ADDED	CAROTENE DESTROYED	CAROTENE DESTROYED
<i>milligram</i>	<i>milligram</i>	<i>per cent</i>
0.029	0.19	66
0.059	0.33	56
0.100	0.62	62
0.234	0.120	51
0.505	0.105	20

tion of Wesson oil and a 0.1 per cent solution of carotene in acetone gave the most nearly homogeneous mixture when 5 ml. of the enzyme mixture was added to it and also gave the most closely reproducible results.

The question of the amount of enzyme and the length of time required for the reaction presented considerable difficulty. One and two ml. of 2.5 and of 1 per cent bean extract were used and neither time nor amount of extract appeared to affect the amount of destruction. However, when graded amounts of from 0.1 to 0.5 ml. of a 1 per cent extract of bean meal were allowed to react for 30 minutes, the percentage of carotene destroyed was found to be related to the quantity of enzyme present.

An experiment was made to ascertain whether the amount of enzyme might better be stated in terms of amounts of carotene destroyed than in percentages. Graded quantities of 0.03–0.50 mg. of carotene in 1 ml. of acetone were pipetted into 200 ml. Erlenmeyer flasks. Five ml. of enzyme mixture containing 1 ml. of soybean meal extract (equivalent to 1 mg. of soybean meal) was added to each flask and the percentage and amounts of carotene destroyed after 30 minutes were determined. As shown by Table 1, the same percentage of carotene was destroyed with all but the 0.5 mg. A homogeneous mixture could not be obtained with 0.5 mg. of carotene. It was therefore concluded that the results are best expressed in percentage.

## METHOD OF ANALYSIS

The method of analysis described below was found to give reproducible results for determination of the carotene-bleaching activity of a series of legume seeds.

*Carotene solution.*—0.1 gram of crystalline carotene, obtained from the S.M.A. Corporation is purified by solution in 2 ml. of  $\text{CHCl}_3$  and precipitation with 25 ml. of ethanol; 50 mg. of the freshly purified carotene is added to 300 mg. of Wesson oil and 400 ml. of acetone in a 500 ml. volumetric flask. The solution is boiled on a water bath until all the carotene dissolves, cooled, and made up to volume with acetone. The solution is kept in a brown bottle in the refrigerator. To insure against some carotene crystallizing from solution, the bottle is heated in a water bath before use and cooled to room temperature.

*Buffer solution.*—The buffer solution is the same as recommended by Sumner and Sumner,<sup>8</sup> 2.36 grams of disodium phosphate and 2.27 grams of potassium acid phosphate per 100 ml. This has a pH of 6.5.

*Preparation of extract.*—0.25 gram of finely ground bean meal is ground in a mortar with 1 or 2 ml. of water. Several more ml. of water are added, and the meal is again ground. The ground meal and water mixture is transferred to a 250 ml. volumetric flask, which is then made up to about 200 ml. The mixture is allowed to stand 2 hours with occasional shaking. It is then made up to volume and mixed, and about 50 ml. is centrifuged at 2400 r.p.m. for 15 minutes. 1 ml. of the extract = 1 mg. of bean meal.

*Procedure.*—The enzyme mixture is prepared by pipetting 2 ml. of the bean extract, 2 ml. of buffer solution, and 6 ml. of water into a test tube and mixing; 1 ml. of carotene solution is pipetted into a 200 ml. Erlenmeyer flask and 5 ml. of the enzyme mixture, equivalent to 1 mg. of bean meal, pipetted down the sides. Do not shake or rotate. The mixture, without shaking, is placed in the incubator at 35°C. for 30 minutes; 100 ml. of U.S.P. ether is added; and the flask is stoppered and shaken until the water layer is colorless.

A blank determination is made on 5 ml. of 4:1 water-buffer mixture added to 1 ml. of carotene solution. The residual carotene in the ether extracts is determined by means of a photoelectric colorimeter and the percentage bleached by the enzyme is calculated according to the equation  $a - b/a \times 100 = \text{per cent destroyed}$ , in which  $a = \text{p.p.m. of carotene in the standard extract}$  and  $b = \text{p.p.m. of carotene in the extract of the sample}$ . These results were at first expressed in percentages of carotene destroyed but, as explained below, they were later expressed as units of carotene oxidase.

## RESULTS

By the use of this method, the percentages of carotene destroyed by 1, 3, and 5 mg. of 17 different samples of beans were determined. These results are given in Table 2. In most cases the first two analyses of the same sample checked well within 10 per cent. With but two exceptions a third analysis gave two results that checked within this per cent of error.

In order to ascertain the relation between the quantities of carotene oxidase in the beans and the percentages of carotene destroyed, the butter bean extract was taken as a preliminary standard. The percentage of carotene destroyed was used as the ordinate, the volume of extract as the

TABLE 2.—*Carotene bleached by different quantities of bean meals*

LABORATORY NUMBER		1 MG.	3 MG.	5 MG.
		BLEACHED	BLEACHED	BLEACHED
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
58846	Butter bean	18	30	46
58847	Lima bean	18	34	47
61836	Baby lima bean	21	45	56
62213	Speckled bush lima bean	29	53	58
58845	Pinto bean	43	61	68
61839	Pinto bean	49	64	68
61834	Pea bean	51	62	68
61837	Red kidney bean	48	65	69
61838	Great northern bean	51	61	66
62210	Black wax bean	44	61	65
61835	Black-eyed pea bean	59	72	80
62211	Black-eyed pea bean	60	73	80
62212	Tennessee crowder	60	75	80
62209	English pea	58	71	79
61840	Laredo soy bean	62	73	80
62214	Laredo soy bean	62	74	81
62334	Mandalay soy bean	72	81	85

abscissa, and the percentages of carotene destroyed by 0.1, 0.3, and 0.5 ml. of butter bean extract were plotted and a curve drawn. The percentage of carotene destroyed by 0.1 ml. of each of the other bean extracts was located on this curve and the corresponding position was found on the abscissa. From the values so found for 0.1 ml., points were marked in the abscissa for the 0.3 and 0.5 ml. of each of the beans and then the percentages of carotene destroyed by these quantities of each bean extract were plotted on the diagram.

TABLE 3.—*Units of carotene oxidase corresponding to percentage of carotene destroyed*

CAROTENE DESTROYED	CAROTENE OXIDASE	CAROTENE DESTROYED	CAROTENE OXIDASE
<i>per cent</i>	<i>units/mg.</i>	<i>per cent</i>	<i>units/mg.</i>
5	0.48	33	4.64
8	0.77	35	5.16
10	0.96	38	5.88
13	1.28	40	6.44
15	1.50	43	7.49
18	1.90	45	8.16
20	2.16	48	9.33
23	2.67	50	10.00
25	3.00	55	14.07
28	3.58	60	21.11
30	4.00		

All the results combined to form a nearly smooth curve, the first part of which is shown in Figure 1. The entire curve slopes steeply up to 50 per cent carotene destruction, then levels off rapidly as the quantity of oxidase increases. There was no evidence of more than one kind of oxidase.

In order to express results in terms of carotene oxidase instead of butter

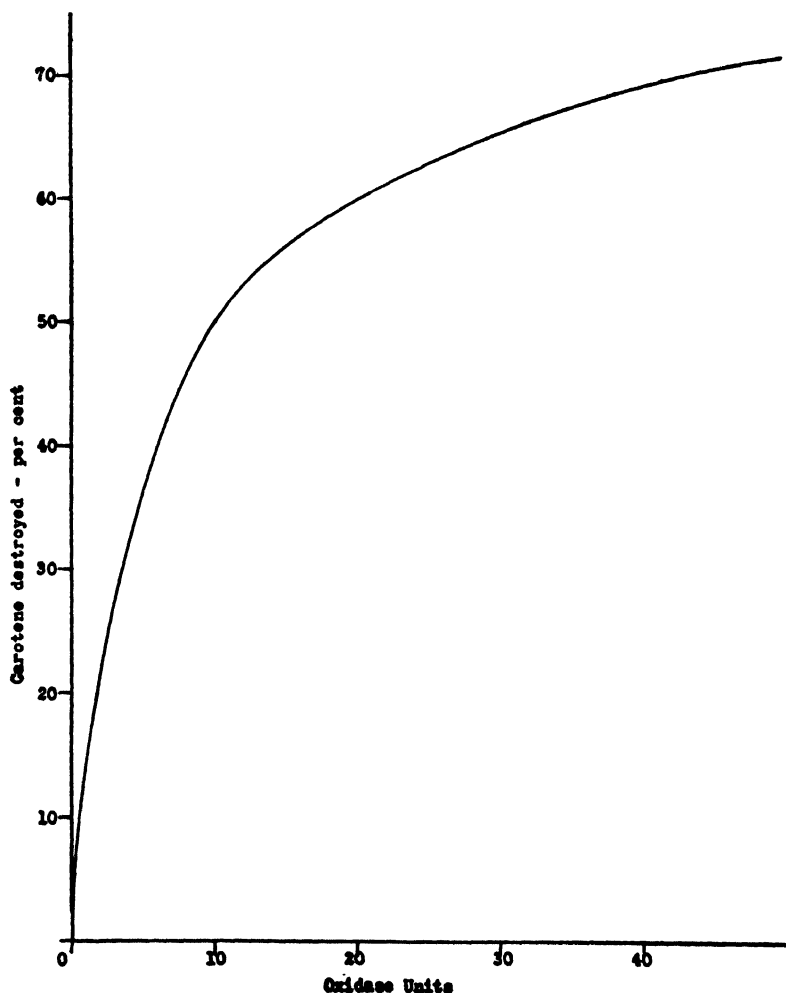


FIG. 1.—RELATION BETWEEN NUMBER OF CAROTENE OXIDASE UNITS AND PERCENTAGE OF CAROTENE DESTROYED.

bean extract, the amount of carotene oxidase that would destroy 50 per cent of the carotene was defined as 10 units of carotene oxidase. Figure 1 is plotted in terms of carotene oxidase so defined and can be used to convert percentage destruction of carotene to terms of carotene oxidase units.

TABLE 4.—*Carotene oxidase activity of a number of legumes*

LABORATORY NUMBER		CAROTENE OXIDASE
		<i>units/mg.</i>
58847	Lima bean	1.9
58846	Butter bean	1.9
61836	Baby lima bean	2.3
62213	Speckled bush lima bean	3.7
63759	Austrian winter pea	5.8
63760	White marrowroot pea	7.3
58845	Pinto bean	7.5
62210	Black wax bean	7.8
63750	Mammoth melting pea	8.1
63773	Navy bean	8.4
61837	Red kidney bean	9.3
61839	Pinto bean	9.7
61834	Pea bean	10.8
61838	Great northern bean	10.8
63757	Tall telephone pea	12.5
63758	Early Alaska pea	13.0
63773	Golden wax bean	13.3
63774	Prolific black wax bean	14.1
63759	Premium gem pea	14.6
63749	American wonder pea	16.2
63753	Red valentine pea	16.6
63756	Dwarf telephone pea	17.1
62209	English pea	18.3
63751	Little marvel pea	19.0
63762	Green split pea	19.7
61835	Black eyed pea	19.7
63752	Nott's excelsior pea	21.0
62211	Black eyed pea	21.1
62212	Tennessee crowder	21.1
63754	Thomas laxton pea	23.3
62214	Laredo soy bean	25.0
61840	Laredo soy bean	25.0
63755	Bliss everbearing pea	25.0
62334	Mandalay soy bean	60.0

After the percentage of carotene destroyed by 1 ml. of the bean meal extract has been determined as described above, the units of oxidase per mg. of bean meal may then be read directly from the curve but for greater accuracy Table 3 should be used. If the percentage of destruction is greater than 50 the accuracy of the determination is considerably reduced because of the leveling off of the curve. In that case it is advisable to repeat the analysis with half or one fourth the amount of legume meal extract previously used and multiply by 2 or 4 to find the units in 1 mg.

The units of carotene oxidase in a number of samples is given in Table 4. The carotene oxidase of the samples is seen to vary from 1.9 to 60.0 units per mg. The lowest quantities are found in the lima beans and the highest in the soy beans. Different samples of the same kind of beans contain approximately the same amounts of oxidase.

### DISCUSSION

One of the most important factors in obtaining reproducible results in the determination is making a homogeneous mixture of extract, buffer, oil, carotene, and water. If too much oil or carotene is used, the mixture floats on the surface and, therefore, is not acted on by the enzyme. Large globules of oil also dissolve carotene and prevent the action of the enzyme.

Another factor was that of aeration. Attempts were made to follow the procedure of Peterson, Hughes, and Scott,<sup>6</sup> who carried on the reactions in test tubes, but because of the small surface the amount of destruction was small and not reproducible. Some of the difficulty may have been due to inability to obtain a homogeneous substrate mixture with the proportions of oil, carotene, and water used by these investigators. Stirring and shaking had a pronounced effect on the rate of destruction, but it was found difficult if not impossible to standardize the degree of aeration by such a procedure. It was finally decided to use a large and constant surface. A 200 ml. Erlenmeyer flask was found to suit the purpose admirably, since 1 ml. of substrate and 5 ml. of enzyme mixture little more than covers the bottom. Furthermore, such a vessel makes extraction of the residual carotene quite simple, it being necessary only to add 100 ml. of ethyl ether and shake for a few seconds. All the carotene goes into the ether layer, leaving the small water layer clear.

Other experiments showed that samples of ground lima beans, pinto beans, and butter beans stored for over a year assayed slightly less than did fresh samples, the difference being well within the limits of error. Ground and unground samples stored for 8 weeks did not lose oxidase. Cooking six samples of the beans in water for 30 minutes entirely destroyed the oxidase.

### SUMMARY

The conditions under which the percentage of destruction of carotene is related to the quantity of carotene oxidase in legumes were ascertained. Based on this relationship a method was devised for determining the quantity of carotene oxidase present. The quantity of carotene destroyed increased rapidly with increases in the quantity of carotene oxidase added when the destruction of carotene was less than 50 per cent of the quantity

<sup>6</sup> Abstracts, Meeting of St. Louis American Chemical Society (1941).

present, but decreased slowly with increases in carotene oxidase beyond this point. The results are shown in a curve, from which the number of units of carotene oxidase may be found from the data on the per cent carotene oxidized under the conditions of the procedure, ten units being defined as the quantity that oxidizes 50 per cent of the carotene in 30 minutes. The carotene oxidase content ranged from 1.9 units per milligram of lima beans to 60.0 units per milligram of Mandalay soy beans.

## PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS

By MAX H. HUBACHER (Laboratory of Ex-Lax, Inc.,  
Brooklyn, N. Y.)

The quantitative method for the determination of phenolphthalein in chocolate preparations described in the 5th edition of *Methods of Analysis*, A.O.A.C., in which phenolphthalein is reacted to the tetraiodo compound, is without doubt the best procedure available. In the course of years, however, the following slight improvements have been worked out and tested in this laboratory:

(1) The fat is removed from the sample by dissolving it with  $\text{CCl}_4$ . Palkin considered the preliminary extraction of the fat with petroleum benzin in an extraction thimble but came to the conclusion that this method "affords an unsatisfactory solution of the difficulty, as a small quantity of phenolphthalein is apt to be carried down with the fat solution." It has been found that the fat can be removed by dissolving it with  $\text{CCl}_4$ , phenolphthalein being insoluble in this solvent. The sample is contained in a Gooch crucible instead of in a thimble.

(2) The iodine reagent is subject to variations in concentration, depending on the analyst preparing it, as the procedure calls for "enough strong potassium hydroxide solution to discharge the iodine color." When  $\text{KOH}$  is added to the iodine solution, the color changes from brown to yellowish but does not turn completely colorless even on addition of an excess. However, it becomes colorless on standing. To avoid these variations, a 0.5  $N$  iodine solution is made with a definite quantity of  $\text{KOH}$ .

(3) It is not necessary to heat the water containing the tetraiodo compound in suspension in order to coagulate the precipitate. The cold solution can be filtered immediately after precipitation, as it filters as fast as when previously heated.

(4) In the improved method outlined at the end of this paper, it was found that it was unnecessary to wash the final tetraiodophenolphthalein in the Gooch crucible with petroleum benzin in order to wash out traces of fat.

(5) Mention should be made in the procedure that the alkaline phenolphthalein solution should be reacted to the tetraiodo compound within 2 hours, as it is not stable and is slowly oxidized by the air.<sup>1</sup> An analyst, after dissolving the phenolphthalein in alkali solution, might leave this solution standing overnight before iodating. In such a case, low results would be obtained.

The following results were obtained when 0.1000 gram of phenolphthalein (U.S.P. XII grade) was dissolved in 1 ml. of 5  $N$  sodium hy-

<sup>1</sup> *This Journal*, 8, 541 (1925).

<sup>2</sup> M. H. Hubacher, U. S. Patent 1,940,495;

dioxide, the solution permitted to stand for various periods of time at room temperature in an open beaker, and then analyzed:

#### PHENOLPHTHALEIN FOUND

(av. of 3 determinations)

hours	per cent
3	9.84
6	9.59
8	9.58
16	9.15
24	8.45
48	8.45

In order to determine the accuracy and the precision of the improved method, two mixtures were made from different lots of phenolphthalein and chocolate. These mixtures were prepared to contain exactly 10 per cent phenolphthalein (U.S.P. XII grade) and 90 per cent chocolate (of 40 per cent fat content). Each mixture was then analyzed 15 times by A. Horner and the writer by the procedure given below:

<i>Phenolphthalein found and mean deviation</i>		
	Analyzed by A. H.	Analyzed by M. H. H.
	per cent	per cent
Mixture 1	$9.89 \pm 0.07$	$9.92 \pm 0.05$
Mixture 2	$9.98 \pm 0.08$	$9.89 \pm 0.08$

The average of all 60 determinations is  $9.92 \text{ per cent} \pm 0.08$ .

The complete procedure incorporating the above suggestions is as follows:

#### METHOD USED

##### REAGENTS

(a) *Iodine solution*.—0.5 N. Dissolve 12.7 grams of KI in 10 ml. of water, add 6.35 grams of iodine, and when dissolved add 12 ml. of 5 N KOH and water to the 100 ml. mark.

(b) *Sodium sulfite solution*.—Dissolve 12.6 grams of anhydrous  $\text{Na}_2\text{SO}_3$  and dilute to the 100 ml. mark.

(c) *Sodium hydroxide*.—5 N.

##### DETERMINATION

Weigh 1 gram of shavings, equal to 0.1 gram of phenolphthalein, into a Gooch crucible (with asbestos pad or fritted glass disk). Extract the fat with 5+4+3 ml. of  $\text{CCl}_4$ , using slight suction towards the end. Place the crucible on a bell jar arrangement or a Witt filter apparatus. Extract the phenolphthalein from the sample by several portions of hot alcohol, collecting the filtrate in a 300 ml. beaker (Berselius or tall form). Wash the underside of the crucible free of phenolphthalein with hot alcohol (using 30–45 ml. altogether). Evaporate the combined alcoholic solution on a water bath. Dissolve the residue at room temperature in 1–1.5 ml. of 5 N NaOH, add a piece of ice (25 grams) and 7–8 ml. of the iodine reagent. Add conc. HCl dropwise from a buret, using a stirring rod, to complete precipitation. If sufficient iodine reagent has been added, the precipitate, as well as the supernatant liquid, will be brown from the excess iodine; if not, add more of the iodine reagent to insure an excess. Dissolve the precipitate again by adding dropwise, with stirring, 5 N NaOH



from a buret. (The resulting solution should be of blue to blue-purple color.) Repeat the process of precipitating and redissolving in the 5 *N* NaOH three more times, adding a small piece of ice if necessary to keep the solution cold. Then add 1–1.5 ml. of the Na<sub>2</sub>SO<sub>4</sub> solution to the blue alkaline solution and filter through a Gooch crucible, or a hardened filter paper, into a 250 ml. beaker, washing several times with water. Acidify the filtrate with HCl, collect the white to greyish precipitate on a weighed Gooch crucible, wash with water until free of Cl, and dry the tetraiodophenolphthalein to constant weight at 110°–130° C.

Weight of precipitate  $\times 0.3872$  = weight of phenolphthalein.

#### REMARKS

The alkaline phenolphthalein solution (but not the tetraiodophenolphthalein solution), is unstable in the air and should be reacted to the tetraiodo compound within two hours. Otherwise, the results will be too low.

The tetraiodophenolphthalein gives off iodine at around 255°–265°C. without melting.

The accuracy of the method is 99.2 per cent with a mean deviation of  $\pm 0.8$  per cent.

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### EFFECT ON FLASKS OF CORROSION BY HYDRO- FLUORIC ACID FROM PHOSPHATES USED IN FERTILIZER ANALYSES

By T. L. OGIER (Texas Agricultural Experiment Station,  
College Station, Texas)

Rock phosphate contains about 3 per cent fluorine, superphosphate about 1.5 per cent, and mixed fertilizers contain amounts corresponding to the superphosphate present. When the fertilizers are dissolved in acids, hydrofluoric acid is liberated. This action has a corrosive effect upon the glass of the graduated flasks in which the solution is made, and in the course of time it will affect the capacity of the flasks.

To determine the extent of corrosion on flasks by hydrofluoric acid, nineteen 200 ml. volumetric flasks that had been used in the determination of total phosphoric acid were selected for testing. These flasks appeared to be badly etched.

Morse's pipets, calibrated by the National Bureau of Standards, were used for testing 200 ml. flasks. With the lower stem graduated from 0 to 100, each graduation represented .02 ml. The correct volume was marked on the pipet used at the 50 mark on the graduated stem. The method used in testing the flasks is as follows:

Connect the Morse pipet with an aspirator bottle containing distilled water. Fill the pipet with distilled water, empty, run distilled water into the pipet above the graduation mark on the upper stem of the pipet, and then allow the water to run out slowly until the meniscus is just resting on the mark. Place the flask to be tested, previously cleaned and dried, under the outlet of the pipet, and run the

water from the pipet into the flask until just below the graduated mark on the neck of the flask, then very slowly until the meniscus rests on the mark on the flask. Record the reading on the graduated stem of the pipet, which will be plus or minus according to whether the water on the stem is below or above the mark 50 on the stem. Refill the pipet and test the other flasks in the same manner. The following table gives the tests of 19 flasks:

FLASK NO.	READING ON STEM	DIFFERENCE IN VOLUME ml.	FLASK NO.	READING ON STEM	DIFFERENCE IN VOLUME ml.
1	65	+ .30	11	46	- .08
2	43	- .14	12	47	- .06
3	47	- .06	13	44	- .12
4	43	- .14	14	48	- .04
5	51	+ .01	15	52	+ .04
6	51	+ .02	16	49	- .02
7	51	+ .02	17	45	- .10
8	54	+ .08	18	70	+ .40
9	65	+ .30	19	70	+ .40
10	70	+ .40			

The results of the tests show that 5 of the 19 flasks had been etched beyond the tolerance allowed, namely  $\pm .20$  ml. in  $\pm 200$  ml. volumetric flask.

## NOTES

### Procedure for Establishing Purity of Volatile Fatty Acids\*

The determination of individual volatile fatty acids in mixtures following the procedure previously published in *This Journal*, 25, 176, is based on distillation data obtained on pure acids. The purity of the acids employed in the work was established by a distillation procedure herein described.

It has been shown (*Ibid.*, 21, 684) that when a dilute solution of a volatile acid is steam-distilled at constant volume in the distilling flask and at a regulated rate of distillation, a definite percentage of the acid will be distilled in any given volume of distillate. It was further shown that if a series of distillates from a volatile acid solu-

TABLE 1.—*Distillation data on volatile acids*

ACID	ORIGINAL DISTILLATIONS		REDISTILLATIONS PROGRESSIVE SUMMATIONS OF PERCENTAGES ON 50 ML. DISTILLATE PORTIONS OF ORIGINAL DISTILLATES		
			1st	2nd	3rd
Acetic	ml.	(19.50)*			
	1st 100	34.75	19.48	19.11	19.07
		(47.50)	35.15	34.67	34.40
	2nd 100	57.48	47.94	47.33	47.00
	3rd 100	72.31	58.11	57.56	57.22
Propionic			—	—	—
		(34.00)	34.00	34.43	33.33
	1st 100	56.03	56.17	56.48	56.19
		(70.60)	70.78	70.74	71.43
	2nd 100	80.55	80.51	80.78	81.90
N-Butyric	3rd 100	91.11	—	—	—
	1st 50	48.25	49.41	49.15	48.90
	2nd 50	73.12	74.08	73.83	73.58
	3rd 50	85.97	86.58	86.51	86.20
		(92.70)	93.05	93.10	92.75

\* Figures in ( ) taken from distillation curve.

tion is collected and titrated, and progressive summations of the percentages of acid distilled are plotted on semi-log paper against volumes of distillate, a straight line will be obtained if the acid employed was pure.

Based upon these considerations the following method was devised for establishing the purity of volatile acids.

#### PROCEDURE

Place a mark on the boiler flask of the distillation apparatus previously described at the 1500 ml. level, fill to this mark with boiled distilled water (boiled distilled water should be used throughout the work), heat to boiling, and boil for several minutes before starting a distillation. Transfer a quantity of the acid to be tested (formic, acetic, propionic, butyric, or isobutyric) that will contain the equivalent of ca. 100 ml. of 0.1 *N* acid to the distilling flask of the apparatus, make to a volume

\* By Fred Hillig, Food and Drug Administration, Federal Security Agency, Washington, D. C.

of 150 ml. with water, add 1 drop of  $H_2SO_4$  (1+1), and heat to boiling. Connect the distilling flask to the boiler and steam-distill under the conditions previously specified.<sup>1</sup> With formic, acetic, or propionic acid collect three 100 ml. portions of distillate, titrating each portion separately. On semi-log paper plot the progressive summations of the percentages of acid distilled, against the volumes of distillate. A straight line indicates the acid is pure. In order further to verify the purity of the acid, transfer the first 100 ml. portion of distillate to the distilling flask and adjust the volume to 150 ml. with water. Make acid with the  $H_2SO_4$  against Congo paper and again distill, collecting four 50 ml. portions of distillate and titrating each separately. The curve plotted from the progressive summations of the percentages of acid distilled in this series should, within experimental error, coincide with the original distillation curve if the acid is pure. Proceed likewise for the second and third 100 ml. portions of distillate. In the case of N-butyric and isobutyric acids, collect three 50 ml. portions of distillate from the original acid in the distilling flask, and on the redistillations collect four 50 ml. portions.

In Table 1, some typical data are given on acetic, propionic, and N-butyric acids. It is emphasized that these data are applicable only with the distillation apparatus by which they were obtained. However, apparatus constructed according to the previous instructions should give approximately the same distillation rates.

### Colorimetric Determination of Lactic Acid\*

A colorimetric method for the determination of lactic acid was described in detail in *This Journal*, 25, 257. Since in the method the lactic acid in the solution in which the color is developed is present as the barium salt, in order to be consistent throughout the procedure for preparing the standard curve required in this determination has been changed and now specifies barium lactate as the standard lactate solution instead of the lithium lactate solution. The revised procedure follows in detail:

Dissolve in ca. 10 ml. of water a quantity of a pure lactic acid salt, such as lithium, zinc, or calcium lactate, that will contain the equivalent of ca. 300 mg. of free lactic acid. Transfer the material to the extractor, add 0.5 ml. of  $H_2SO_4$  (1+1), and adjust the volume to 50 ml. Extract with ether for 3 hours. Add ca. 20 ml. of water to the extraction flask, evaporate the ether on the steam bath, and carefully titrate the contents of the flask with 0.1 N  $Ba(OH)_2$ . Transfer the neutralized material to a 200 ml. volumetric flask, make to mark, and shake. Pipet into a 500 ml. volumetric flask such a quantity of this barium lactate solution as will contain the equivalent of 250 mg. of free lactic acid, make to mark, and shake. This barium lactate solution is employed in preparing the standard curve. Use the quantities of solution given in Table 1 in the previous publication (*loc. cit.*), following the procedure outlined except to add 6.6 ml. of 0.1 N HCl to the 50-55 ml. volumetric flask during the carbon treatment instead of the 3.3 ml. previously specified. With barium lactate a total of 6 ml. of 0.1 N HCl is required in the Nessler tube. The 40 ml. of filtrate from the carbon treatment will contain 4.8 ml. Therefore, add 1.2 ml. of 0.1 N HCl to the 40 ml. aliquots in the Nessler tube.

In the application of the method for lactic acid when the standard curve based on barium lactate is used, 6.6 ml. of 0.1 N HCl is required in the 50-55 ml. volumetric flask during the carbon treatment and a total of 6 ml. of the acid is required in the Nessler tube, as provided in the procedure for preparing the standard curve.

### Colorimetric Reaction for Arsenate in Arsenite Solutions†

It is sometimes necessary in work on the eradication of ticks to test the arsenate content of very dilute (about 0.04 N) arsenite solutions. High precision of the test is not required. It is sufficient to know whether 1, 2, 5, or 10 per cent of the arsenic is present in the pentavalent form.

Pentavalent arsenic in strongly acid solutions releases free iodine from iodide, and this reversible reaction will be complete if the iodine is fixed by another reagent.

\* By Fred Hillig, Food and Drug Administration, Federal Security Agency, Washington, D. C.

† By F. L. Hahn, University of Minnesota, School of Chemistry, Minneapolis, Minn.

If fluorescein is used for that purpose, the color change of the dyestuff will indicate the quantities of arsenate present. Evidently this test, being an oxidation reaction, is not strictly specific for arsenate; but the arsenite solutions will hardly contain another oxidant except possibly traces of iron. The effect of iron can be eliminated by acidifying the solutions with an excess of phosphoric acid.

#### REAGENTS

*Fluorescein*.—Dissolve 0.1 gram of fluorescein in water, add a few drops of NaOH and 50 grams of IK, and dilute to 100 ml.

*Arsenite*.—Dissolve 1 gram of  $As_2O_3$  in 20 ml. of normal NaOH and dilute to 500 mg.

*Arsenate*.—Acidify 10 ml. of the arsenite solution, add bicarbonate, and titrate with iodine; add the volume of iodine solution found by this trial to 100 ml. of the arsenite. This solution contains 10% of the arsenic in the pentavalent form; by mixing it with the arsenite solution, solutions with a lower content of arsenate are prepared.

*Phosphoric acid*.— $D = 1.5$ ; 66.6% of  $H_3PO_4$ .

#### DETERMINATION

To 1 ml. of the solution to be tested add 0.2 ml. of fluorescein and 0.2 ml. of the  $P_2O_5$  (or 5 drops of each) and heat in a boiling water bath for 15 minutes. The color of the solution changes according to the content of arsenate from a greenish yellow to a brownish yellow and further to a reddish brown; 0.5% is detectable by a skilled analyst; 1% is very marked, and the higher percentages are easily distinguishable.

In the laboratory the test can be made more sensitively by shaking the solution with 2 ml. of ether to extract the dyestuff. Comparison scales can be prepared with standard solutions in sealed tubes or by color prints.

## BOOK REVIEWS

**Chemistry of Insecticides and Fungicides.** By DONALD E. H. FREAR, D. Van Nostrand Company, Inc., 250 Fourth Avenue, New York. 1942. 300 pages. Price \$4.00.

This book is an outgrowth of a course of lectures on the chemistry of insecticides and fungicides given by the author over a period of several years, and he has brought together a wealth of material.

The chapters dealing with organic insecticides are important, and most of the recent work on this class of compounds is included. In another chapter many of the newer fumigants, including the aerosols of Sullivan, Goodhue, and Fales, are mentioned. There is a good description of the older insecticides and fungicides, such as arsenicals, sulfur, and copper compounds and oil emulsions, and also a good chapter on spray deposits or spray residue.

On page 12 the statement is made that the use of calcium arsenate in this country is declining. This is not correct, since calcium arsenate is the standard insecticide for use against the cotton boll weevil.

Several materials are mentioned, p. 69, as activators for pyrethrum sprays, but the one that is probably most extensively used—isobutyl undecyleneamide—is not mentioned. However, this material is mentioned later, p. 194, under the heading "Synergists."

Sixty-eight pages are devoted to methods of analysis, a large number of which are reprinted from "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," 5th Edition. All of the methods available from this source are not included, and in some instances the author has made poor selections. For instance, the method given for determination of lead in lead arsenate, p. 217, is one intended for use in the analysis of a complicated mixture such as Bordeaux-lead arsenate-zinc arsenite. The A.O.A.C. method for rotenone in derris and cube powders is given under the heading "Derris and Cube Preparations," and it is not suitable for *all* of these preparations.

At the end of each chapter is appended an extensive list of references. In several instances these are not strictly accurate. For example, credit for the mercury reduction method for determination of Pyrethrin I in pyrethrum powder, p. 225, is given to Holaday, who only modified it, and no mention is made of Wilcoxon, who originated it. Also in connection with the method for determination of rotenone, p. 229, no mention is made of Jones, the senior author.

Notwithstanding the above criticism, the book will be of great help to those interested in the chemistry and use of insecticides and fungicides, and will prove to be a welcome addition to the library of such workers.—J. J. T. GRAHAM.

**The Application of Absorption Spectra to the Study of Vitamins, Hormones, and Coenzymes,** 2nd Ed. By R. A. MORTON, Department of Chemistry, The University of Liverpool. 1942. Adam Hilger, London, England. 1942. 226 pages. 14×21.5 cm. Price, \$6.50.

In spite of its small size (226 pages), this book covers especially well the subject of vitamins, and it also deals with such interrelated fields as hormones, proteins, purine, and pyrimidine derivatives, enzymes, and coenzymes.

The main purpose of the book is to show the place of absorption spectrophotometry in establishing the basic facts in the various fields covered as well as to indicate whenever possible its specific utility for assay purposes.

Although the vitamin chapters are quite summarized, they are thorough and they also are just about as up-to-date as could be expected in such a rapidly expanding sphere of investigation.

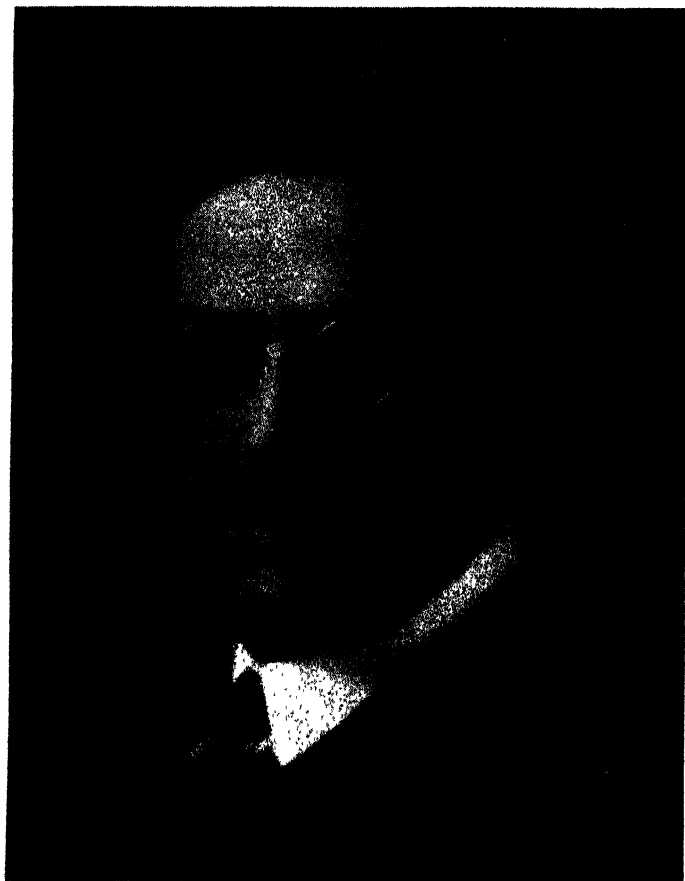
The treatment of the vitamins appears to be much more nearly complete than some of the other subjects considered. For example, the chapter on provitamins and vitamin A has 53 pages and the B complex, 51 pages, while the chapter on proteins is discussed in only 5 pages.

The author has been closely associated with the work to establish the extinction coefficient of vitamin A and a consequent conversion factor for calculating biological potency. Hence it is not surprising that one of the more interesting discussions is concerned with this problem. Unfortunately some of the more recent and more thorough of the American work dealing with this problem apparently was not available to the author so that his pertinent conclusions cannot be regarded as authoritative as might have otherwise been possible. Also, on page 98 of related discussion, the statement, "by using the conversion factor  $E_{1\%}^{1\text{cm}}$  328,  $I = 1600$ , the potency in respect of vitamin A can be obtained in I.U./gm" is somewhat loose and is not consistent with the author's own preceding conclusions.

The book should not be misjudged because of the above-mentioned deficiencies. It is a valuable book. The spectroscopic viewpoint provides essentially a practical working viewpoint and as such it should be prized by workers in any of the fields covered. Also, since most of the broader and more essential points are so ably summarized, the book should be desirable for reference, or to any chemist who wishes to further his knowledge of vitamins without extensive reading. Adequate references are given for more detailed study.—J. B. WILKIE.







CARL LUCAS ALSBERG, 1877-1940

## CARL LUCAS ALSBERG

Carl Lucas Alsberg was a scientist and philosopher. His interests covered a wide range. His exceptional intellectual endowments combined with his marvelous memory enabled him to reach quickly the outposts of knowledge on any subject he studied. His insatiable curiosity impelled him to explore the unknown and so extend the horizons of knowledge in those fields that intrigued him—and most fields did intrigue him.

In an address to a graduating class two years before his death, Alsberg said:

I am an example of arrested development, for I have never lost the curiosity that we, all of us, have as little children who drive their elders half crazy by constantly asking them—why? Most of us, as we grow up, cease asking why. I didn't. And so all my life my consuming interest has been research—asking, what is it? why is it? and what of it?

After telling the young men and women of the class that specialization is the road to follow if they would seek fame or fortune, he continued:

But I've done even worse than fail to be a specialist: I haven't even stuck to the career I started on. Physician, biochemist, teacher, chemist, administrator, economist—I've had a fling at all of them in turn. It's been fun, but it hasn't made a great man of me. Be warned by me and stick to your "last," if it's fame you want or material success. But remember there's a price to be paid for this kind of success—a price not to be measured in tangible things but rather in the number of one's friends, the narrowness of one's outlook, the character of one's taste, one's capacity for understanding and enjoyment. One may in old age be bored and lonely. I recommend that you consider well the cost before you decide upon the easy career of the narrow specialist.

Alsberg was born on April 2, 1877, in New York City. He was graduated from Columbia University with an A.B. degree and later earned an A.M. degree. From the College of Physicians and Surgeons of the same university he received, in 1900, an M.D. degree. During the next two years he studied in Germany at the Universities of Strassburg and of Berlin. Following his studies in Germany he became an instructor in biological chemistry at Harvard. He accepted, in 1908, an appointment as a biological chemist in the Bureau of Plant Industry, U. S. Department of Agriculture, where for four years he did research work under Dr. Rodney H. True.

I well remember my first meeting with Alsberg in the lounge of the Cosmos Club when he first came to Washington. We were both interested in the affairs of the local section of the American Chemical Society, and through this frequent contact there developed a mutual understanding and regard that continued through the years without interruption. After his appointment in December, 1912, as Chief of the Bureau of Chemistry, I came to know him intimately, and for the succeeding nine years I was closely associated with him. The announcement of his appointment came as a surprise to many. His name had been mentioned for the place, but it was generally expected that some member of the staff of the Bureau of Chemistry with experience in food and drug law enforcement would be appointed.

Alsberg was selected for this important post by President Taft. The President considered it wise to appoint a man not involved in any of the controversies that had resulted from differences of opinion within the Department of Agriculture as to the best way to solve food and drug law enforcement problems. Alsberg was recommended to the President by a number of the leading chemists and other scientists throughout the Nation.

The Bureau of Chemistry, at the time of Alsberg's appointment as Chief, was engaged in investigational work in agricultural and industrial chemistry and on the

analytical and inspectional work necessary for the enforcement of the Federal Food and Drugs Act. There was a departmental Board of Food and Drug Inspection to make administrative decisions and a Board of Consulting Scientific Experts to settle scientific controversies. Both boards were abolished shortly after Alsberg's appointment, and he was given authority to direct all phases of food and drug law enforcement operations.

Alsberg, who had lived all his adult years in the cloistered shelter of universities or in the serene atmosphere of research laboratories, suddenly found himself in the center of controversies that periodically reached cyclonic intensity. He was without administrative training or experience, yet there were thrust upon him for solution administrative problems of the utmost complexity. He had still to learn the capabilities of the various members of his staff well enough to know upon whose judgment he could rely. A less resourceful man than Alsberg would have been appalled.

With superb confidence Alsberg undertook the impossible task of personally going to the bottom of every pressing problem that confronted him. He soon learned, however, that this was futile, as he sensed that the element of time, which is inconsequential in basic research work, is the essence of good administration. After a thorough study of the problem he reorganized the bureau, separating the research work from the regulatory activities. He placed in key positions men in whose judgment he had confidence and detailed to them authority to make all but the most important decisions. He created the district and station organization for the field staff engaged in law enforcement operations. He established additional research laboratories in Washington. Upon completion of the reorganization, which required several months, he was able to give more of his personal attention to the research work, which from the first had enlisted his chief interest.

Even then Alsberg's insatiable curiosity led him to keep many irons in the fire. Years later in the same commencement address previously quoted, Alsberg pointed out that "Goat-feathers," as expounded by Ellis Parker Butler, are the distractions, side lines, and defections that take a man's attention from his own business and keep him from getting ahead. "Goat-feathers," said Alsberg, "look very well in Who's Who or in your obituary, but they interfere with your work. I know from experience, for I lack the necessary force of character to say no. It's only because for years I've had hard-boiled secretaries, both men and women, that I've been able to get anything at all done. So again be warned by me."

Notwithstanding Alsberg's modest disparagement of his achievements, few bureau chiefs in the Federal service have accomplished more with comparable facilities and in a similar period than he accomplished during the nine years he was Chief of the Bureau of Chemistry.

David F. Houston, who was Secretary of Agriculture during most of the time Alsberg was a bureau chief, had great confidence in his judgment. The Secretary gave Alsberg a free hand in organizing and directing the work of the Bureau of Chemistry and frequently consulted him about scientific problems arising elsewhere in the Department. It became routine procedure for the Secretary's office to refer to Alsberg for comment correspondence on certain subjects originating in any bureau of the Department.

On his own initiative Alsberg frequently made constructive recommendations to the Secretary about not only the work of the Department but also about scientific work in other Federal agencies, as he was concerned lest the overlapping activities would react adversely to all scientific work of the Federal Government.

Alsberg placed the research work of the Bureau of Chemistry on a high plane and also recommended that an agency be established to coordinate the scientific work of the entire Federal service. He expanded the work directed towards improving methods for processing, preserving, and transporting foods. He had the vision to

see that one of the greatest services that chemistry can render is the discovery of new ways to utilize products of the farm, and with only meager means he started utilization projects that became highly productive. He inaugurated studies in biological chemistry that contributed much to the solution of food and drug law enforcement problems.

Insisting that research to be productive must be done by men and women with research aptitude and vision, Alsberg considered it a waste of money and of man power to assign to research problems routine workers with a card-index type of mind, but he was lenient to a fault with those members of his staff who revealed as much as a glimmer of aptitude for research. Personnel problems stemming from this characteristic brought him no end of embarrassment. Those who recall some of the personnel incidents that plagued him while he was chief of the bureau will appreciate this additional quotation from his commencement address of many years later:

As I look back upon my own training I realize that one of the most valuable things I got from the study of medicine is a little knowledge of psychiatry. Time and again it has kept me from doing an injustice by making me realize that a given personnel problem was not a matter for discipline or a court martial but rather for an alienist.

Alsberg took an active interest in the work of the A.O.A.C. He was elected Secretary of the Association in 1914 and served for six years. He established the *Journal* of the Association in order that the proceedings and methods might be more currently available. The first number appeared in 1915. It was on his recommendation also that the methods are now published in book form rather than as a Department bulletin. Other constructive suggestions made by him were adopted by the Association.

When the United States entered the World War in April, 1917, Alsberg immediately placed the facilities of the Bureau of Chemistry at the service of the War Agencies. Every division and laboratory in the bureau was soon at work on the solution of some war problem. Various members of the staff were detailed to aid the War and Navy Departments. Alsberg personally served on no less than fifteen war committees. He assisted in organizing the Gas Warfare Service and directed laboratory investigations for that service. He was called into frequent consultations by Herbert Hoover, who was the U. S. Food Administrator. Alsberg's extensive knowledge of foods, chemistry, biology, and medicine, together with his facility in clear expression and his sound judgment, made him one of the most sought-after consultants in the Government service.

After the close of the war, Alsberg became convinced that the economy program made necessary by the huge war debt would make it impossible for many years to obtain adequate appropriations to finance the research projects in which he was so deeply interested. He resigned in 1921 from the Government service to become a Director of the Food Research Institute at Stanford University. In that position he directed projects that have been of great value to various food industries and to the Nation. He served also as Dean of Graduate Study at Stanford and as a member of the Social Science Research Council. He was chairman of the Pacific Coast Committee of that Council.

Alsberg, in 1937, accepted the Directorship of the Giannini Foundation of Agricultural Economics at the University of California. His new duties included direction of the Agricultural Research Center at the university and membership on the National Resources Planning Board. He expressed gratification to personal friends that at the age of sixty, when many men are ready to retire, he could take on a new position and begin a career as an economist, a profession new to him.

On one of his trips to the East in October, 1940, while in Washington, Alsberg developed a severe cold. He immediately took a train to California. En route he was

stricken with pneumonia, but continued his journey. When he reached Berkeley he was rushed to a hospital. The disease was too far advanced for effective treatment. Within a few hours he was dead.

His death was a great loss to science. His attainments were respected among scientists throughout the world. He was an accomplished linguist and kept abreast of the scientific literature of many countries. He was a delightful conversationalist, a clear and forceful writer, and a magnetic public speaker. He is missed in the scientific world, but most of all he is missed by those of us who not only admired his intellectual attainments and his sterling qualities of character, but who from intimate association also had a sincere personal affection for Alsberg, the man.

W. W. SKINNER

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## REPORT ON PHOSPHORIC ACID

### FACTORS AFFECTING THE AVAILABILITY OF AMMONIATED SUPERPHOSPHATES, PART I\*

By JOHN O. HARDESTY, WILLIAM H. ROSS (*Associate Referee*), and  
J. RICHARD ADAMS (Bureau of Plant Industry, Washington, D. C.)

The water-insoluble phosphates in ammoniated mixtures differ as a rule from those in the ordinary types of non-ammoniated mixtures in that they show greater differences in their solubility in citrate solution. As a result of these differences in citrate solubility, the official method may sometimes indicate a higher availability for a water-insoluble phosphate in low than in high analysis mixtures, and in mixtures than in the undiluted material (12). This defect in the method becomes increasingly objectionable with increase in the ammoniation of the mixture and its concomitant increase in the water-insoluble phosphates formed in the reaction.

Owing to the recent greatly increased capacity of the United States for producing synthetic ammonia, the cost of this form of nitrogen is likely to be still further reduced following the war. It would therefore seem to be of considerable economic importance to know not only the maximum quantity of free ammonia that can be added to a fertilizer mixture without reducing the availability of the phosphorus to plants, but also to have an accurate means for evaluating such a mixture.

In accordance with a recommendation (11) that was approved last year, a further study was undertaken of (1) the factors that affect the chemical availability of the  $P_2O_5$  in ammoniated mixtures; (2) the efficiency of ammoniated mixtures in promoting crop growth; and (3) improved means for the more accurate evaluation of such mixtures.

The present paper is limited to a study of the factors that affect the chemical availability of the  $P_2O_5$  in ammoniated mixtures. Data on the availability to plants of ammoniated mixtures of varying composition and on proposed methods for the more accurate evaluation of such mixtures will be presented in subsequent papers.

### FACTORS AFFECTING REVERSION OF AMMONIATED SUPERPHOSPHATES

It is known that the rate and extent of the reversion of  $P_2O_5$  in an ammoniated superphosphate, as determined chemically by the official method, depend on such factors as the degree of ammoniation (5, 6, 12, 14), the rate at which the mixture cools following ammoniation (2, 3, 4), and the presence of such component materials as fluorides and dolomite (7, 8, 9, 10). Thus, marked reversion, as determined by the official method for phosphate availability, is known to take place when a heavily am-

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\* Presented in part at the meeting of the American Chemical Society held at Buffalo, N. Y., Sept. 7-11, 1942.

moniated superphosphate-dolomite mixture is slowly cooled from its initial ammoniation temperature (6), but the reversion is much less if the mixture is quickly cooled, particularly in the absence of dolomite (2, 3, 4). Although many of the factors that affect the availability of ammoniated superphosphates are thus fairly well understood, the effect of other factors is not so well understood, and this is particularly true with respect to the effect of the different factors mentioned on the efficiency of ammoniated mixtures. Thus, while it is known that reversion in a heavily ammoniated superphosphate mixture can be decreased by rapid cooling of the mixture, it remains to be demonstrated that the efficiency of the mixture in promoting crop growth can be increased by the same treatment.

The different factors affecting ammoniated superphosphate availability to which special attention is given in this study are (1) the degree of ammoniation of the mixture; (2) the storage temperature of the mixture following ammoniation; (3) the moisture content of the mixture during ammoniation and storage; (4) the sources of the superphosphates used in the preparation of the ammoniated superphosphate mixtures; (5) the presence of dolomite in the mixture during ammoniation and storage; and (6) the presence of fluorides in the mixture during ammoniation and storage.

#### AMMONIATED SUPERPHOSPHATE MIXTURES

The ammoniated samples used in this investigation were prepared in the form of 4-12-4 mixtures. The formulas of the mixtures, when the free ammonia added was varied from 0 to 5 per cent on the basis of the superphosphate present, or from 0 to 60 pounds per ton, are given in Table 1. The total nitrogen content of each mixture was kept constant by the addition of the necessary quantity of ammonium sulfate. The proper quantities of potassium chloride, dolomite, and filler to give a mixture in each case of the desired grade were added to each mixture prior to ammoniation. In the second, third, fourth, and fifth mixtures, the free ammonia was added in the form of solutions of ammonia of such concentrations that the moisture content of each mixture was increased to 9 per cent when the specified quantity of free ammonia had been added. The mixtures were ammoniated in a stainless steel rotating drum mounted on trunnions. Each ammonia solution was sprayed into the mixture in the drum by being forced under pressure through an opening in one of the trunnions of the drum. The ammoniation of each mixture was completed in 3-4 minutes.

In preparing another mixture according to the fifth formula of Table 1, the ammonia was added in the form of anhydrous ammonia to a mixture containing less than 1 per cent of moisture. It was found that a dry mixture of this kind will not absorb anhydrous ammonia until the temperature of the mixture is increased to about 45°C. Once ammoniation started under these conditions it proceeded rapidly with a marked increase in the

TABLE 1.—*Formulas of ammoniated 4-12-4 mixtures containing Superphosphate A*

MATERIAL	POUNDS PER TON					
Superphosphate A, $P_2O_5 = 20.34\%$	1180	1180	1180	1180	1180	1180
Free Ammonia	0	24	36	48	60	60
Ammonium Sulfate, $N = 20.51\%$	390	292	244	195	146	146
Potassium Chloride, $K_2O = 50.12\%$	160	160	160	160	160	160
Dolomite	250	250	250	250	250	0
Filler (sand)	20	94	130	167	204	454
	2000	2000	2000	2000	2000	2000

temperature of the mixture. Its moisture content also increased to 3 per cent due to liberation of the hydrated water in the mixture. All mixtures were kept below a temperature of  $65^\circ\text{C}.$  during ammoniation by the addition of water when necessary to the outside of the drum. Each mixture, on being ammoniated, was passed through a 10-mesh screen and thoroughly mixed.

The mixed samples as prepared were divided into three portions. One portion was quickly cooled and stored at  $20^\circ\text{C}.$ ; the second portion was stored at  $60^\circ\text{C}.$ ; and the third portion at  $90^\circ\text{C}.$  The samples were all stored in large, wide-mouthed bottles for a period of 36 days. Any considerable loss of moisture from the samples during storage was prevented by having a long glass tube pass through the stopper in each bottle and extend for a distance of about 2 feet through the top of the constant temperature chamber in which they were stored. The stored samples were finally air-dried and ground to pass a 40-mesh sieve.

The results obtained in the analysis of the samples are given in Table 2. It will be seen that when the samples were stored at  $20^\circ\text{C}.$ , no increase in

TABLE 2.—*Citrate-insoluble  $P_2O_5$  in ammoniated 4-12-4 mixtures containing Superphosphate A*  
(Initial citrate-insoluble  $P_2O_5 = 0.74\%$ )

AMMONIATION RATE		MOISTURE	DOLomite	INCREASE IN CITRATE-INSOLUBLE $P_2O_5$ IN SAMPLES STORED FOR 36 DAYS AT—		
PER CENT OF SUPERPHOSPHATE	(LBS. $NH_3$ /TON)			$20^\circ\text{C}.$	$60^\circ\text{C}.$	$90^\circ\text{C}.$
		per cent		per cent	per cent	per cent
0	0	9	Present	0.01	—	—
2	24	9	Present	0.01	0.28	2.60
3	36	9	Present	0.01	0.38	2.81
4	48	9	Present	0.07	0.63	2.75
5	60	9	Present	0.19	0.59	1.73
5	60	3	Present	0.00	0.47	0.32
5	60	9	Absent	0.01	0.42	0.26



citrate-insoluble  $P_2O_5$  occurred as determined by the present official method, even at 5 per cent ammoniation. A significant increase in citrate-insoluble  $P_2O_5$  took place when separate portions of the same mixtures were stored at 60°C. and still more when the storage temperature was increased to 90°C. It will be seen that the citrate-insoluble  $P_2O_5$  in the mixtures stored at temperatures above normal was essentially the same at 2 per cent ammoniation as at 5 per cent. This held true with the mixtures prepared from the particular superphosphate used in these experiments. With mixtures prepared from superphosphates obtained from other sources, the citrate-insoluble  $P_2O_5$  was sometimes found to show a marked increase with increase in the rate of ammoniation beyond 2 per cent. That different superphosphates sometimes react differently on ammoniation has frequently been observed in the industry.

Although no appreciable increase in citrate-insoluble  $P_2O_5$ , as measured by the present official method, took place on storage at 20°C. in any of the mixtures represented in Table 2, a considerable reversion was indicated when a 2 gram sample was taken for analysis, as in the old official method, and the extent of the reversion increased with increase in the rate of ammoniation.

Table 2 further shows that no serious reversion occurred in any of the mixtures containing 3 per cent of moisture even when ammoniated to 5 per cent and stored for 36 days at 90°C. The same held true for the dolomite-free mixtures containing 9 per cent of moisture. It would seem, therefore, that the dolomite in association with moisture was responsible for the greater part of the reversion that took place in the mixtures stored at 90°C.

#### AMMONIATED SYNTHETIC SUPERPHOSPHATE MIXTURES

It has been shown by MacIntire and his coworkers (7, 8, 9) that fluorine, as calcium fluoride, reacts with tricalcium phosphate to form fluorapatite and that the same product is also formed when calcium fluoride is included in a mixture of triple superphosphate and a basic material such as limestone. This observation seemed to be confirmed in this laboratory when it was found that the presence of calcium fluoride in an ammoniated monocalcium phosphate sample caused a marked increase in citrate-insoluble  $P_2O_5$  during storage at temperatures above normal (10, 13). The replacement of calcium fluoride with sodium fluoride increases the rate of reaction, and a marked reversion may then take place in the process of making the analysis (10).

It is possible, therefore, that fluorine may play an important part in the fertilizer efficiency of ammoniated superphosphates. A set of fluorine-free and of fluorine-containing synthetic superphosphate samples was accordingly prepared by a procedure similar to that described for the ordinary superphosphate samples. In the preparation of these samples, C.P.

monocalcium phosphate and calcium sulfate were mixed in the proportion of one mol of the former to two mols of the latter to simulate a commercial superphosphate. These mixtures were used in the preparation of 4-12-4 fertilizers, of which one contained no fluorine, a second contained fluorine as calcium fluoride equal to 10 per cent of the  $P_2O_5$  in the mixture, and a third contained the same proportion of fluorine in the form of sodium fluoride. Each of these fertilizers was of two types, one of which was non-ammoniated, while the other was treated with ammonia at the rate of 60 pounds per ton of mixture. The ammoniated mixtures were divided into two parts, of which one was stored for 36 days at  $20^\circ C.$ , and the other for the same length of time at  $60^\circ C.$  The stored mixtures were air-dried and ground to pass a 40-mesh sieve as in the case of the ordinary superphosphate mixtures. The formulas of the mixtures are given in Table 3. All mixtures were dolomite-free.

TABLE 3.—*Formulas of ammoniated 4-12-4 mixtures containing a synthetic superphosphate*

MATERIAL	POUNDS PER TON					
$Ca(H_2PO_4)_2 \cdot H_2O + CaSO_4 \cdot 2H_2O$ , <sup>1</sup> $P_2O_5 = 20.68\%$	1161	1161	1161	1161	1161	1161
Free Ammonia	0	60	0	60	0	60
Ammonium Sulfate, N = 20.51%	390	146	390	146	390	146
Potassium Chloride, $K_2O = 50.12\%$	160	160	160	160	160	160
Calcium Fluoride <sup>2</sup>	—	—	49	49	—	—
Sodium Fluoride <sup>2</sup>	—	—	—	—	53	53
Filler (sand)	289	473	240	424	236	420
	2000	2000	2000	2000	2000	2000

<sup>1</sup> Two mols of  $CaSO_4 \cdot 2H_2O$  to one of  $Ca(H_2PO_4)_2 \cdot H_2O$ .

<sup>2</sup> Fluorine added equals 10% of  $P_2O_5$  in mixture.

No significant increase in citrate-insoluble  $P_2O_5$  was found on analysis in the non-ammoniated mixtures, nor in those that had been ammoniated in the presence of fluorine to 5 per cent of the superphosphate contained therein. As this result might appear to be at variance with the observations of MacIntire and his associates (7, 8, 9) as well as with the previous tests made in this laboratory (10, 13), the work was repeated on various combinations of materials. The formulas of the mixtures are given in Table 4. It will be noted that all mixtures contained the same quantity of  $P_2O_5$  and all were ammoniated to the same degree in relation to the  $P_2O_5$  present. Mixtures 1 and 2 were simply ammoniated monocalcium phosphates; the first contained no fluorine while the second contained fluorine as sodium fluoride equal to 10 per cent of the  $P_2O_5$  in the mixture. The table shows that the sodium fluoride caused a marked increase in citrate-insoluble  $P_2O_5$ , as was to be expected from the previous work done in this laboratory (10). Mixture 3 differed from Mixture 2 in that it contained

TABLE 4.—*Formulas and analyses of miscellaneous fertilizer mixtures\**

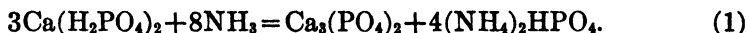
MATERIAL	POUNDS PER TON										
	1	2	3	4	5	6	7	8	9	10	11
						Formulas					
Double Superphosphate	—	—	—	—	—	—	—	—	—	495	495
Monocalcium Phosphate	426	426	426	426	426	426	426	426	426	—	—
Calcium Sulfate	—	—	560	—	—	—	—	560	560	—	—
Free Ammonia	60	60	60	60	60	60	60	60	60	60	60
Ammonium Sulfate	—	—	—	—	—	146	146	146	146	146	146
Potassium Chloride	—	—	—	—	—	160	160	160	160	160	160
Sodium Fluoride	—	53	53	53	53	53	53	53	53	53	53
Dolomite	—	—	—	—	—	250	—	250	—	250	—
Filler	53	—	—	560	1461	905	1155	345	595	836	1086
	539	539	1099	1099	2000	2000	2000	2000	2000	2000	2000
						Analyses					
Increase in citrate-insoluble $P_2O_5$ after 15 days' storage at 20°C.	0.04	6.60	2.00	0.82	none	none	none	0.46	trace	trace	none
Increase in citrate-insoluble $P_2O_5$ after 15 days' storage at 75°C.	0.45	6.44	1.33	1.15	trace	trace	trace	0.90	trace	trace	none

\* Samples 1 and 2 are 9-45-0 mixtures; Samples 3 and 4 are 5-22-0 mixtures; Sample 5 is a 4-12-0 mixture; and Samples 6 to 11 are 4-12-4 mixtures.

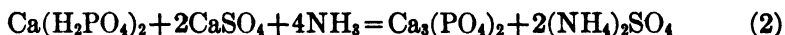
calcium sulfate. In Sample 4 the calcium sulfate of Mixture 3 was replaced with an equal weight of sand, and in Mixture 5 the sand was increased to give a 4-12-0 mixture.

Mixtures 6-11 were ammoniated 4-12-4 mixed fertilizers. In Mixtures 6-9 the phosphatic component was monocalcium phosphate and in Mixtures 10 and 11 it was double superphosphate. It will be noted that none of these mixtures showed any appreciable reversion on storage except Mixture 8, which contained both calcium sulfate and dolomite.

The reactions taking place on the complete ammoniation of monocalcium phosphate may be represented by the equation:\*



When the monocalcium phosphate is accompanied by two mols of calcium sulfate, as in Samples 3, 8, and 9 of Table 4, the complete ammoniation of the mixture takes place by a different reaction, which is commonly represented by the equation:\*



In this equation all the phosphorus in the monocalcium phosphate is changed to tricalcium phosphate, whereas in Equation 1 only one-third of the phosphorus in the mixture is changed to tricalcium phosphate. It would be expected, therefore, that a greater proportion of the total  $\text{P}_2\text{O}_5$  in a completely ammoniated monocalcium phosphate would be changed into the citrate-insoluble form when calcium sulfate is present, as in superphosphate, than when it is absent. Keenen (5) has shown that when a superphosphate is ammoniated to the same degree as the mixtures represented in Table 4, the phosphatic components of the ammoniated product consist of about 3 parts of tricalcium phosphate to 1 part of monocalcium phosphate. The product so obtained is acid in reaction. According to White, Hardesty, and Ross (14), the phosphatic components formed under the same conditions in the absence of any considerable proportion of calcium sulfate, as in a double superphosphate, consist of about 2 parts of dicalcium phosphate and approximately 1 part each of mono- and diammonium phosphates. This product is neutral or alkaline in reaction and loses ammonia on storage. It would therefore be expected that the presence of dolomite would increase the reversion in acid mixtures that contain calcium sulfate, such as Mixture 8, but not in neutral or alkaline mixtures, such as Mixtures 6 and 10, which do not contain calcium sulfate.

The citrate-insoluble  $\text{P}_2\text{O}_5$  in all the mixtures represented in Table 4 was determined by the official method, and a 1 gram sample was used in each case. It will be seen that the  $\text{P}_2\text{O}_5$  in a 1 gram sample of Mixture 2 is nearly four times as great as in Mixture 5. Because of this difference in

\* It is recognised that more basic phosphates than tricalcium phosphate may be formed in these reactions.

dilution, a given quantity of any form of  $P_2O_5$  in the two mixtures will represent a percentage nearly four times greater in Mixture 2 than in Mixture 5. It will likewise follow that the same relationship will hold true between the ratio of  $P_2O_5$  to the citrate solution used in the analysis of the mixtures. It seemed possible, therefore, that the dilution factor alone might explain the difference in the results found for citrate-insoluble  $P_2O_5$  in Mixtures 2 and 5, and this was confirmed when an analysis of Mixture 2 diluted after storage with the same proportion of sand as in Mixture 5 showed no appreciable citrate-insoluble  $P_2O_5$ . This indicates that if Mixture 2 contained fluorapatite, a like quantity was also present in all the mixtures that contained the same proportion of fluorine, ammonia, and monocalcium phosphate.

The present official method for determining citrate-insoluble  $P_2O_5$  has been generally accepted as a satisfactory procedure for the evaluation of non-ammoniated mixtures. It is also considered to be fairly well adapted to the evaluation of ammoniated mixtures of average  $P_2O_5$  content. However, the results given in Table 4 and those now being obtained in a study of the availability of ammoniated mixtures to plants indicate that the method is unsatisfactory for ammoniated mixtures of varying  $P_2O_5$  content, as previously pointed out (12). If it is assumed that the present official method is satisfactory for ammoniated mixtures of average  $P_2O_5$  content, the results given in Table 4 indicate that high values are obtained for available  $P_2O_5$  in ammoniated mixtures of low  $P_2O_5$  content and low values for those of high  $P_2O_5$  content.

#### SUMMARY

A study was made of the chemical availability of  $P_2O_5$  in ammoniated fertilizer mixtures as affected by the degree of ammoniation, the storage temperature following ammoniation, the moisture content during ammoniation and storage, the sources of superphosphates used in the preparation of the mixture, and the presence of dolomite and of fluorides during ammoniation and storage.

Mixtures containing commercial superphosphates from different sources varied greatly with respect to increase in insoluble  $P_2O_5$  when the degree of ammoniation was increased beyond 2 per cent. There was no appreciable increase of citrate-insoluble  $P_2O_5$  during storage of these mixtures at 20°C. The results of tests in which identical ammoniated products were stored for 36 days at temperatures of 60° and 90°C. indicate that the presence of dolomite in association with sufficient moisture was responsible for such reversion as occurred at storage temperatures above normal. Similar tests were conducted with ammoniated and non-ammoniated mixtures containing synthetic superphosphates prepared from C.P. monocalcium phosphate and calcium sulfate with and without additions of fluoride compounds. The mixtures that simulated a 4-12-4 grade of fertilizer without dolomite contained no significant amounts of citrate-

insoluble  $P_2O_5$  even after ammoniation in the presence of fluorides at the rate of 60 pounds of ammonia per ton and storage in a moist condition for two weeks at  $75^\circ C$ .

Treatment of the same quantity of monocalcium phosphate as that contained in the 4-12-4 mixture with the same quantity of ammonia as that used in ammoniating the mixture gave only a slight increase of citrate-insoluble  $P_2O_5$  on storage of the ammoniated sample at  $75^\circ C$ ., but this same procedure in the presence of fluorides caused a marked increase in the citrate-insoluble  $P_2O_5$  content, as would be expected.

When this procedure was carried out on the same quantities of materials, but diluted with sand to give a mixture simulating a 4-12-0 fertilizer, no appreciable citrate-insoluble  $P_2O_5$  was detected in the ammoniated product stored at  $20^\circ$  and only a trace at the storage temperature of  $75^\circ C$ . The presence of calcium sulfate in the synthetic 4-12-4 mixture produced an acid condition in the ammoniated product and, in this medium, dolomite increased the reversion of  $P_2O_5$  to an appreciable extent. However, in neutral or alkaline mixtures not containing calcium sulfate the dolomite did not increase reversion.

These findings demonstrate the effect of dilution on the result obtained for citrate-insoluble  $P_2O_5$  when determined by the official method on a 1 gram sample. This dilution factor holds true with respect to the quantity of  $P_2O_5$  present in a 1 gram sample as well as for the ratio of  $P_2O_5$  to citrate solution used in the analysis.

If it is assumed that the present official method for citrate-insoluble  $P_2O_5$  is satisfactory for ammoniated mixtures of average  $P_2O_5$  content, the results obtained in this study indicate high values for available  $P_2O_5$  in ammoniated mixtures of low  $P_2O_5$  content and low values for those of high  $P_2O_5$  content.

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## REPORT ON MINERAL MIXED FEEDS\*

By ALFRED T. PERKINS, *Associate Referee*, and J. F. MERRILL  
(Kansas Agricultural Experiment Station, Manhattan, Kan.)

The official (first action) method for the determination of calcium in mineral feeds (*Methods of Analysis*, A.O.A.C., 1940, 365, 48) does not give complete recovery of the calcium under all conditions. A number of the many mineral feeds on the market contain the soluble orthomonocalcium phosphate ( $\text{CaH}_4(\text{PO}_4)_2$ ), and some of these feeds do not yield all of their calcium by this method. The difficulty seems to be that under acid conditions certain calcium-phosphorus mixtures, when ignited, produce an insoluble calcium phosphate. This phosphate is probably a metaphosphate, and the reaction probably is  $\text{CaH}_4(\text{PO}_4)_2 + \text{heat} \rightarrow \text{Ca}(\text{PO}_3)_2 + 2 \text{H}_2\text{O}$ . This calcium phosphate is insoluble in acids but apparently under conditions as outlined in the method it is not produced in the presence of an excess of a nonvolatile alkali.

The Associate Referee's attention was first called to the difficulty by B. P. Sutherland of The Consolidated Mining and Smelting Company and by George E. Grattan of the Ottawa Department of Agriculture. Test analyses were made on feed samples submitted, and it was decided to re-check the official method.

## TEST PROCEDURE

A series of mineral mixtures rich in calcium or phosphorus was tested. These mixtures were made from orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) and calcium carbonate ( $\text{CaCO}_3$ ). The chemicals were mixed in such proportions that pure monocalcium, dicalcium, and tricalcium orthophosphates were formed as well as mixtures of these compounds with each other and the acid or base. The molecular ratios in the various mineral mixtures are outlined in Table 1, which also shows the calculated amount of calcium present when determinations were made on each mixture. Each mixture was made up separately. The calcium carbonate was weighed into a porcelain dish and ignited, and the diluted phosphoric acid was pipetted in slowly, to allow the calcium oxide and the phosphoric acid to react.

The official method (loc. cit.) is essentially as follows: A 2.0 gram sample is ignited, dissolved in HCl (1+3), neutralized and calcium precipitated, and titrated.

The method apparently gives low results when the calcium-phosphorus ratio is such as to give monocalcium dihydrogen orthophosphate, under which condition the calcium is rendered insoluble in the acid used in the second step.

\* See descriptions of Methods A, B, C, D, E, and F previously given. Methods G and H are same as E and F with organic matter added to sample.

TABLE 1.—*Calcium-phosphorus ratios tested and calculated quantity of calcium precipitated*

MIXTURE NUMBER	MOLECULAR RATIO $H_2PO_4-CaCO_3$	THEORETICAL COMPOUNDS PRESENT AND PROPORTIONS	CALCULATED INORGANIC CALCIUM*	
			SERIES A-F	SERIES G & H
			gram	
1	4-1	$2H_2PO_4+CaH_4(PO_4)_2$	0.0163	0.0016
2	4-2	$CaH_4(PO_4)_2$	0.0271	0.0027
3	4-3	$CaH_4(PO_4)_2+2CaHPO_4$	0.0347	0.0035
4	4-4	$CaHPO_4$	0.0405	0.0041
5	4-5	$2CaHPO_4+Ca_3(PO_4)_2$	0.0449	0.0045
6	4-6	$Ca_3(PO_4)_2$	0.0485	0.0049
7	4-7	$4Ca_3(PO_4)_2+CaCO_3$	0.0514	0.0051
8	4-8	$4Ca_3(PO_4)_2+2CaCO_3$	0.0538	0.0054

\* Corrected for calcium in organic material.

The method given above and the variations tested are outlined below.

A. The official method (see above).

B. Extremely slow cooling after ignition in order to test the possibility of the formation of a more soluble crystal.

C. Substitution of HCl-HNO<sub>3</sub> (5+1) for HCl-H<sub>2</sub>O (1+3).

D. Substitution of HCl-HNO<sub>3</sub> (1+5) for HCl-H<sub>2</sub>O (1+3).

E. Mixing of the sample with Na<sub>2</sub>CO<sub>3</sub> (2+15) before igniting.

F. Ignition omitted, mineral treated directly with HCl-H<sub>2</sub>O (1+3).

Methods E and F were also tested with 90% organic matter in the sample.

The results of the above tests are listed in Table 2.

TABLE 2.—*Calcium recovery (per cent) from various calcium-phosphorus mixtures when analyzed by A.O.A.C. method and variations*

MIXTURE NUMBER	METHOD							
	A* A.O.A.C. METHOD	B SLOW COOLING	C ASH DIS- SOLVED IN HCl-HNO <sub>3</sub> (5+1)	D ASH DIS- SOLVED IN HCl-HNO <sub>3</sub> (1+5)	E IGNITED WITH Na <sub>2</sub> CO <sub>3</sub> ; DISSOLVED IN HCl (1+1)	F NOT IGNITED	G SAME AS E+90% ORGANIC MATTER	H SAME AS F+90% ORGANIC MATTER
1	64.5	34.6	57.0	13.0	101.1	99.1	100.2	98.5
2	20.2	27.2	20.1	23.4	100.2	99.1	100.3	100.0
3	62.9	72.6	78.4	73.3	101.3	98.7	100.0	100.7
4	96.7	92.2	94.7	90.1	101.2	98.7	96.9	99.4
5	96.5	97.1	97.9	96.5	100.6	98.8	102.2	100.0
6	96.7	97.8	98.2	97.8	97.7	99.1	103.6	100.5
7	96.6	97.4	94.6	98.7	100.3	98.9	103.9	101.0
8	97.0	98.6	99.8	98.1	100.2	98.7	102.8	100.0

\* Contribution No. 278, Department of Chemistry.



## DISCUSSION

The results obtained indicate that the official (first action) method does not return all the calcium when the acidity of the sample is such that monocalcium dihydrogen phosphate forms. The calcium can be recovered either by omitting the ignition or igniting under alkaline conditions. If the ignition is omitted, the calcium that is present in any organic matter will not be returned and thus the calcium precipitated will show the quantity of added mineral calcium in the feed rather than the total calcium. However, as the method is essentially for mineral feeds and added mineral calcium, this differentiation is of little import. If the mineral mixture is ignited with excess sodium carbonate, the excess base seems to prevent the formation of the insoluble metaphosphate.

## REPORT ON STARCH IN RAW AND BAKED CEREALS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory,  
State College, Miss.), *Associate Referee*

The Hopkins revision of the Mannich-Lenz procedure<sup>1</sup> for the determination of starch in cereals was studied in 1941 by the Association, but only three collaborators reported (*This Journal*, 25, 621). Owing to the promise of this method, the Associate Referee decided to continue the study this year, and in the case of the raw cereals to compare the polarimetric method with the Munsey modification of the Rask procedure (*Methods of Analysis*, A.O.A.C., 1940, 221).

The following samples were selected for study: No. 1, sweetpotato starch; No. 2, whole wheat flour; No. 3, corn flakes; and No. 4, whole wheat bread.

The sweetpotato starch was manufactured by the Laurel Starch Plant in Mississippi. The sweetpotato is not considered a cereal, but the Southern Regional Laboratory had asked that this starch be included in the investigation because it is a new product, and is considered to be worthy of study. The whole wheat flour was a two to one mixture commonly used by most bakeries, and the bread was the resultant product. The corn flakes were Post Toasties. All samples were ground in a ball mill to pass a 100-mesh sieve and were allowed to come to laboratory humidity before being sent out. Therefore all results are given on an "as is" basis.

The samples were sent to eleven collaborators with instructions to analyze the sweetpotato starch and the whole wheat flour by both the Rask and Hopkins procedures, but as the Rask method was not applicable to baked cereals, the corn flakes and the whole wheat bread were to be

<sup>1</sup> *Can. J. Research*, 11, 751-758 (1934).

analyzed only by the Hopkins method. If a polarimeter was not available and additional time was available the collaborators were requested to try the samples by the diastase-hydrochloric acid procedure (*Ibid.*, 359). In view of the filtering difficulties encountered in previous studies of the Hopkins procedure, all analysts were asked to use any available filtering aid. Where two sets of results were submitted by the collaborator, only the one from the accelerated filtering was used in the tables.

Results were received from seven collaborators, whose efforts make this report possible. If more than four results were given on a sample by any one method, only the four best results were used in order to save space in the report. The list of collaborators follows:

V. E. Munsey, Food and Drug Administration, Washington.

E. F. Snyder and Mr. VonKorff, Northern Regional Research Laboratory, Peoria.

J. D. Guthrie and E. T. Steiner, Southern Regional Research Laboratory, New Orleans.

C. O. Willits and W. L. Porter, Eastern Regional Research Laboratory, Philadelphia.

The W. E. Long Company, Chicago.

B. A. McClellan, General Mills, Minneapolis.

American Dry Milk Institute, Chicago.

M. P. Etheredge.

#### DISCUSSION OF RESULTS

Owing to the wide variation in results, no attempt was made to record accepted averages on the samples by the different methods. The widest variation was shown in the results by the Rask procedure on the sweet-potato starch, and the least variation was shown in results on this same sample by the Hopkins polarimetric method. The next greater extent of variation was in the results on the corn flakes by the diastase-hydrochloric acid method. Again the Hopkins procedure gave good checks. The whole wheat bread gave wider variations by the Hopkins procedure than by the diastase-hydrochloric acid method. It should be borne in mind that this is the most difficult sample to filter, and that this is a factor in the polarimetric method. Also, if the averages of the highest and lowest results on this sample be omitted, very close checks would be shown. This would have been true also in the case of the results from the diastase-hydrochloric acid method on the corn flakes. All analysts check themselves and each other reasonably well on the whole wheat flour by all three methods. The good checks on the whole wheat flour and its bread by the diastase-hydrochloric acid method bear out a former statement to the Association that this may be the best for low starch cereals (*This Journal*, 24, 113).

The Hopkins and diastase-hydrochloric acid methods gave about the same percentage of starch on the whole wheat flour and the whole wheat bread. However, in the case of the sweetpotato starch and the corn flakes

TABLE 1.—*Starch results on sweetpotato starch by three methods*

ANALYST	RASK MODIFIED METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE-HYDROCHLORIC ACID METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	71.80	92.00	87.40
	74.50	91.80	87.00
			88.20
Av.	73.15	91.90	87.53
2		90.60	86.10
		90.65	85.70
			85.90
			86.10
Av.		90.63	85.95
3	79.03	90.34	
	80.99	90.33	
	80.12		
Av.	80.05	90.34	
4	87.01	90.54	
	86.58	90.00	
	86.92	90.85	
Av.	86.84	90.46	
5	83.72	92.93	82.53
	83.64	92.59	82.98
	83.14	92.54	82.53
	83.88	90.12	83.07
Av.	83.60	92.05	82.78
6	87.26	90.37	
	86.10	90.47	
Av.	86.68	90.42	
7	89.16		86.29
	88.64		87.13
	87.86		86.17
	87.52		85.61
Av.	88.30		86.30
8	81.60	90.43	
	81.50	90.34	
	79.92	90.25	
Av.	81.01	90.34	

TABLE 2.—*Starch in whole wheat flour by three methods*

ANALYST	RASK MODIFIED	HOPKINS POLARIMETRIC	DIASTASE-HYDROCHLORIC ACID
	METHOD	METHOD	METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	60.30	58.80	60.10
	60.30	59.20	60.30
	—	—	—
Av.	60.30	59.00	60.20
2		59.30	58.50
		59.10	58.70
	—	—	—
Av.		59.20	58.60
3	62.87	60.94	
	63.12	60.87	
	62.85		
	—	—	—
Av.	62.95	60.91	
4	60.32	58.80	
	61.97	58.17	
	61.02	57.95	
	—	—	—
Av.	61.10	58.31	
5	59.28	60.13	59.31
	59.32	61.29	59.22
	59.44	60.13	59.27
	59.46	60.77	59.31
	—	—	—
Av.	59.38	60.58	59.28
6	61.00	58.12	
	60.10	57.45	
	—	—	—
Av.	60.55	57.79	
7	61.44		61.60
	61.28		61.31
	61.24		60.70
	—	—	—
Av.	61.32		61.20
8	59.10	56.62	
	59.50	56.84	
	—	—	—
Av.	59.30	56.73	

TABLE 3.—*Starch in baked cereals*

ANALYST	CORN FLAKES		WHOLE WHEAT BREAD	
	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD	HOPKINS POLARIMETRIC METHOD	DIASTASE- HYDROCHLORIC ACID METHOD
1	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	74.40	67.80	55.70	55.00
	74.10	66.40	56.00	54.70
		66.40		54.50
Av.	74.25	66.87	55.85	54.73
2	73.40	68.30	54.95	54.70
	73.60	68.90	54.80	54.50
				54.40
Av.	73.50	68.60	54.88	54.53
3	72.28		53.37	
	72.17		53.63	
			53.42	
			53.36	
Av.	72.23		53.45	
4	72.02		51.10	
	73.38		50.88	
	72.73		50.65	
Av.	72.71		50.88	
5	74.65	57.69	55.46	54.36
	74.39	57.83	54.46	54.32
	74.78	57.65	54.12	54.27
	74.65	57.38	54.33	54.68
Av.	74.62	57.69	54.59	54.41
6	75.08		57.08	
	75.23		57.25	
Av.	75.16		57.17	
7		65.82		58.39
		65.59		57.43
Av.		65.70		57.91
8	73.09		54.68	
	73.17		54.16	
	73.17		54.51	
Av.	73.14		54.45	

the Hopkins method gave higher results. In fact, on a dry basis the results for the sweetpotato starch are over 100 per cent by all participating analysts (average moisture 9.99 per cent). This would indicate that the specific rotation of 200 for wheat starch is not satisfactory for other starches and that it would be necessary to work out a different specific rotation for each starch before the polarimetric method could be generally used.

### MISCELLANEOUS RESULTS

Referee Munsey tried the Rask procedure on the corn flakes and bread and obtained very low results, confirming his previous work and the fact that the procedure is not applicable to baked cereals. He also tried the direct acid hydrolysis with first washing as under the polarimetric method. The results on the corn flakes and the sweetpotato starch were reasonably in line with 71.40 per cent and 87.50 per cent, respectively, while the results on the wheat products were very high (flour 66.40 per cent, bread 60.20 per cent), showing that it would be necessary first to treat these products with diastase.

The only new method brought to the attention of the Association was that for the rapid determination of starch in crude gluten, by Clendenning.<sup>2</sup> It consists in removing interfering proteins from the dispersion with stannic chloride. Clendenning later adjusted this method to whole and white wheat flours,<sup>3</sup> but it was received too late to be tried out by the Association this year.

### COMMENTS BY COLLABORATORS

*Analyst 1.*—The manipulation of the A.O.A.C. diastase method is improved by washing, as under the polarimetric procedure, for the removal of sugars. This procedure, or some modification of it, seems to be the best method for general application.

*Analyst 2.*—Cetyl alcohol was used to cut down foaming in the Hopkins procedure. For filtering, a No. 5 Whatman filter paper was used; it folded over at each 90°, as described by Treadwell-Hall. When only 10% alcohol was used for washing in the diastase procedure, the leachings were blue with iodine.

*Analyst 3.*—The result on the sweetpotato starch by the Rask procedure was raised from 80.05 to 87.81% by allowing to stand 30 minutes instead of 5 minutes. Suction speeded up filtering in the Hopkins procedure but gave higher results owing to evaporation loss. However, if Celite is added and the mixture centrifuged, there is no difficulty in filtering. The Hopkins method is preferred to the Rask procedure.

*Analyst 5.*—In the Hopkins procedure filtering was aided considerably by putting the 100 ml. flask in boiling water for 5–10 minutes and again cooling before making up to mark. In the diastase-hydrochloric acid method, the strength of the malt infusion should be doubled.

<sup>2</sup> *Can. J. Research*, 20, 403 (1942).

<sup>3</sup> Unpublished report.

*Analyst 6.*—A jet of air controlled foaming when samples were boiled with the calcium chloride solution.

The Associate Referee believes that this year's work confirms previous indications of the fact that both the diastase-hydrochloric acid and the Hopkins procedures offer possibilities for starch determinations. With the lack of definite specific rotations for the individual starches, the diastase method may be more generally applicable than the polarimetric method. However, in spite of the specific rotation, the results on the sweetpotato starch indicate that the Hopkins method is no more in error than is the diastase-hydrochloric acid method. It is believed that both methods warrant further comparative study.

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### REPORT ON ASH

By J. L. ST. JOHN (Division of Chemistry, Agricultural Experiment Station, Pullman, Wash.), *Associate Referee*

Further collaborative work was done to determine the applicability of the Association's method for ash (*Methods of Analysis*, A.O.A.C., 1940, 354) and to compare results obtained at other temperatures, including 550° and 600°C. Four samples were submitted to each of four different groups of collaborators. They were asked to determine the ash on these samples by the official method at 650° and also at 550° and 600°C. Sixteen authentic samples, described in the heading to Table 2, were analyzed. Each lot of feed was very thoroughly mixed before being bottled for shipment. It was suggested that the collaborator empty each sample from the bottle on receipt and thoroughly mix before making the analysis. Reports from 27 collaborators are incorporated into Table 1, which presents the average of the duplicate results reported by each collaborator. By request, duplicate determinations were reported to the second decimal place.

The complete results were condensed and correlated as in previous reports. Table 2 shows the results of all collaborators (from Table 1) averaged under each specified condition. While only averages are presented in Table 1, the differences between duplicates presented in the original reports to the Associate Referee were determined and averaged, and they are incorporated into Table 3. The maximum range covered by the collaborators' average results (Table 1) are given in Table 4. The average decrease in the percentage of ash due to increase in temperature, calculated from Table 2, is reported in Table 5. This shows the influence of an increase of 50° in the temperature of ashing on the percentage of ash in each of the samples.

The samples included four types of mixed poultry feeds, a turkey con-

TABLE 1.—*Collaborative results (average)*

COLLABORATOR	550°C.				600°C.				650°C.			
	20	85	89	92	20	85	89	92	20	85	89	92
1. Grady-Baird	11.32	10.77	9.77	1.85	11.11	10.65	9.87	1.83	10.30	10.57	9.11	1.87
2. Entwistle-Bicknell	11.34	10.88	9.67	1.86	10.36	10.61	9.16	1.87	10.18	10.58	8.62	1.84
3. Feinstein-Christie			9.40	1.73			9.12	1.74			8.59	1.83
4. Swift-Curtis	10.80		9.58	1.86	10.22		8.85	1.82	10.10		8.51	1.74
5. Dessyok	11.42	10.78	9.61	1.87	11.20	10.48	9.48	1.71	10.25	10.29	8.76	1.39
6. Kaufman-Ellis	11.35	10.85	9.62	1.93	10.38	10.67	9.26	1.88	10.25	10.52	8.63	1.85
7. Kerr	11.34	10.63	9.57	1.87	10.56	10.46	9.36	1.73	10.30	10.44	9.14	1.75
8. Allen	10.66	10.72	9.07	1.83	10.38	10.67	8.53	1.84	10.19	10.52	8.40	1.85
Average	11.17	10.77	9.54	1.85	10.60	10.59	9.20	1.80	10.22	10.49	8.72	1.76
	26	83	90	93	26	83	90	93	26	83	90	93
9. Ashbury-Fraps	8.87	2.90	5.69	7.30	8.71	2.88	5.48	7.18	8.20	2.76	5.22	6.96
10. Frary	9.0	2.8	5.9	7.7	8.6	2.8	5.7	8.1	7.8	2.8	5.3	7.3
11. Roberts-Frits	9.14	2.92	6.43	7.55	8.87	2.93	5.83	7.60	8.69	2.76	5.74	7.45
12. Nasif-Geagley	8.80	2.80	5.70	7.54	8.66	2.79	5.48	7.64	8.02	2.77	5.27	7.48
13. Hall	8.41	2.80	5.44	7.64	7.90	2.70	5.52	7.50	8.07	2.93	5.49	7.83
14. Sandiford-Hand	8.8	2.8	5.6	7.6	8.4	2.8	5.4	7.6	7.9	2.8	5.4	7.5
15. Flach-Hasbrouck	8.85	3.07	5.66	7.45	8.67	2.90	5.34	7.43	8.10	3.02	5.16	7.23
16. Bunting-Elmslie	8.53	2.82	5.65	7.49	7.94	2.84	5.56	7.47	7.98	2.85	5.62	7.41
Average	8.76	2.86	5.76	7.53	8.47	2.83	5.54	7.55	8.10	2.84	5.40	7.40
	28	84	87	94	28	84	87	94	29	84	87	94
17. Haskins	8.56	10.40	4.57	4.04	8.40	10.20	4.40	3.89	8.20	9.92	4.38	4.11
18. Krober-Hopper	8.32	10.45	4.43	3.94	8.06	10.23	4.38	3.99	7.87	10.02	4.42	3.95
19. Ory-Kishlar	8.18	9.98	4.34	3.86	8.09	9.80	4.34	3.78	7.63	9.62	4.40	3.74
20. McClure	8.30	10.17	4.33	3.82	7.55	9.93	4.34	3.80	7.40	9.07	4.27	3.53
21. Morris	8.4	10.5	4.4	3.8	8.2	10.4	4.4	3.8	7.8	10.1	4.3	3.8
22. Matheeson-Nixon	8.52	10.49	4.45	3.90	8.24	10.25	4.45	3.84	8.20	10.19	4.52	4.32
Average	8.38	10.33	4.42	3.89	8.09	10.14	4.39	3.85	7.85	9.82	4.38	3.91
	29	81	86	88	29	81	86	88	29	81	86	88
23. Randall	12.42	1.33	5.93	3.50	12.42	1.31	5.90	3.40	11.77	1.30	5.90	3.41
24. Randle	12.29	1.83	6.07	3.85	11.43	1.72	5.84	3.60	11.42	1.65	5.75	3.68
25. Struve	12.56	1.36	6.05		11.93	1.34	5.90		11.72	1.38	5.93	
26. Zeigler-Mitchell	12.33	1.38	6.03	3.47	11.58	1.38	5.97	3.47	11.58	1.38	5.95	3.47
27. Dinneen-Alves	12.10	1.34	6.04	3.54	12.08	1.31	5.93	3.47	11.69	1.28	5.83	3.40
Average	12.34	1.45	6.02	3.59	11.89	1.41	5.91	3.49	11.64	1.40	5.87	3.49

centrate, which was higher in protein and ash, and a dairy feed. Two types of the feeding material, straw and silage, had not been used in previous studies. Fish meal from another variety of fish was substituted for herring meal previously used. A fairly wide variety of plant and animal materials utilized in feeding were used during the work on the development of the ash method here recommended.

The data presented confirm the conclusions reached in the earlier reports, showing that the consistency of results is essentially the same at



TABLE 2.—Average of collaborative results on ash (per cent)

TEMP.	LAYING MASH	DOG FISH	ALFALFA	WHEAT	STARTING MASH	
	20	85	89	92	26	
°C.						
550	11.17	10.77	9.54	1.85	8.76	
600	10.60	10.59	9.20	1.80	8.47	
650	10.22	10.49	8.72	1.76	8.10	
	BARLEY	BOY BEAN	STRAW	DEVELOPING MASH	SILAGE	
	83	90	93	28	84	
550	2.86	5.76	7.53	8.38	10.33	
600	2.83	5.54	7.55	8.09	10.14	
650	2.84	5.40	7.40	7.85	9.82	
	MILL RUN	DAIRY FEED	TURKEY CONCENTRATE	CORN	LINSEED	PEAS
	87	94	29	81	86	88
550	4.42	3.89	12.34	1.45	6.02	3.59
600	4.39	3.85	11.89	1.41	5.91	3.49
650	4.38	3.91	11.64	1.40	5.87	3.49

temperatures of 550°, 600°, and 650°C. Table 3 of this report and Table 3 in the previous report (*This Journal*, 25, 861) show that an analyst is capable of checking his own results about equally well, regardless of which

TABLE 3.—Average differences between duplicates (per cent)

TEMP.	20	85	89	92	26	
°C.						
550	.145	.11	.022	.028	.103	
600	.083	.077	.07	.024	.10	
650	.108	.09	.044	.028	.134	
	83	90	93	28	84	
550	.103	.103	.102	.093	.057	
600	.07	.162	.172	.125	.05	
650	.045	.078	.105	.053	.05	
	87	94	29	81	86	88
550	.02	.06	.033	.015	.02	.02
600	.05	.025	.048	.015	.028	.075
650	.015	.025	.02	.025	.005	.023

TABLE 4.—*Maximum range between results obtained by collaborators (per cent)*

TEMP.	20	85	89	92	26	
°C.						
550	.76	.25	.70	.20	.73	
600	.98	.21	.34	.17	.97	
650	.20	.29	.74	.48	.89	
	83	90	93	28	84	
550	.27	.99	.40	.38	.51	
600	.23	.49	.92	.85	.45	
650	.26	.58	.67	.87	1.12	
	87	94	29	81	86	88
550	.23	.24	.46	.50	.14	.38
600	.11	.21	.99	.41	.13	.20
650	.25	.58	.35	.37	.20	.28

of the three temperatures is used. In both of these reports, the results may be slightly in favor of a temperature of 600°C. rather than of either 550° or 650°C. At all temperatures, the overall average difference between duplicates is well below .1 per cent, the average being about .07. From Tables 4 in both of these reports, the maximum range between the results obtained by different collaborators, there seems little choice between temperatures, although the result may slightly favor 600°C. or 550°C. Table 5 further demonstrates a progressive small loss of ash as the temperature of ashing increases. This difference may be slightly less when the

TABLE 5.—*Loss in ash due to increase of ashing temperature (per cent)*

TEMP.	20	85	89	92	26	83	90	93
°C.								
550	.60	.18	.34	.05	.29	.03	.22	+.02
600								
650	.35	.10	.48	.04	.37	+.01	.14	.15
	28	84	87	94	29	81	86	88
550	.29	.19	.03	.04	.34	.04	.11	.10
600								
650	.24	.32	.01	+.06	.15	.01	.04	0

temperature is increased from 600° to 650°C. in comparison with the loss when the temperature is increased from 550° to 600°C.

The available information from collaborative work dealing with the types of dish to be used in the ashing procedure was presented in *This Journal*, 23, 634-5. Collaborators have emphasized that the muffle furnace should not be overloaded, since precise, dependable results cannot be obtained under such conditions. In the eight reports involved in this study, a total of over 90 different, individual collaborators have very generously cooperated, and their data are incorporated in the seven reports that appear in *This Journal*. While the reports by the Associate Referee present the collaborators' results to the second decimal place, it should be emphasized that this is for the purpose of this study only and that analytical reports of the results by the Association's method should be made to first decimal place only, since the work shows that the precision of the method does not justify utilization of the second decimal place.

#### DISCUSSION

It has been assumed (*This Journal*, 24, 848) that the primary objective of the ash determination is to demonstrate the relative amount of mineral matter present in the sample. Including the present paper, the eight reports describing this work appear to emphasize the need for a standardization of the method for ash determination. The different reports, particularly those by St. John (*This Journal*, 25, 857), St. John and Midgley (*Ibid.*, 24, 932), and St. John and Midgley,<sup>1</sup> indicate a closer relationship existing between the percentage of ash and the total mineral matter present when lower temperatures are used, in comparison with higher temperatures.

The results in the various reports, particularly those presented by St. John (*This Journal*, 25, 969) are believed to demonstrate that the previous criteria for the determination of "completeness of ashing" are not justified. In general, the need for a standardized method is demonstrated. To compare results, either for the purposes of investigation or for control work, each analyst needs to adhere carefully to the details of the established standardized procedure. Occasionally modified procedures are utilized, the defense in each case being some specialized reason. While the need for adaptability in the method may in some cases be evident and desirable, great caution should be exercised in attempting to establish any modification, particularly in analytical methods of this general type. The question of modification of the temperature or time of ashing when calcium carbonate is included in mixed feeds has frequently been raised. This was previously discussed in these reports (*This Journal*, 25, 863). Not only would lower temperatures seem to give a closer correlation with the total

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<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 301 (1942).

amount of mineral matter present, since the total carbon dioxide in carbonates is an integral part of the calcium carbonate, but the additional amount of carbon dioxide that is lost when ashing is done at higher temperatures undoubtedly has no relation to the feeding value.

A standardized uniform procedure for ashing is desirable; it should take into consideration as far as possible the various needs and requirements. The details of this procedure should be carefully adhered to in making ash determinations. If it should be demonstrated at any time that the accepted and approved procedure does not adequately meet the situation with respect to a specific product the applicability of the method should be studied with a view to its restandardization.

The Associate Referee requested an opinion from the collaborators regarding a choice between an ashing temperature of 600° and 650°C., and requested suggestions on other features of the method. The many collaborators responding indicated a preference in the ratio of about 5 to 1 for a temperature of 600°C.

The Committee on Recommendations of Referees (*This Journal*, 25, 47) recommended that the temperature of ashing be changed to 600°C., official (first action). With the additional data available in the present report, the Associate Referee recommends that the temperature of 600°C. be made official (final action). This lower temperature was also discussed in *This Journal* 24, 848; 25, 863.

The ash method has been very widely utilized in preparing many types of samples for analysis to determine the percentage of the mineral elements present. One of the results of the present investigation is an emphasis of the need for a further investigation of the ash method when used for this purpose. Many of the results reported in the present series of papers were from a variety of plant material and the method appears applicable for use on plant material other than feeds. Due to the variation in the percentage of ash with the temperature at which the ashing is done and the accompanying loss of certain mineral elements, it would appear that certain fields that have based the determination of mineral elements on the ashing procedure would need to be reworked. Also it may be undesirable to express the percentage of mineral elements on an ash basis. It would seem to be more logical to determine the total amount of mineral elements present in plant and certain other organic samples by a wet ashing procedure, such as the perchloric acid digestion, expressing the percentage on the dry sample basis. In the case of potassium, for instance,<sup>1</sup> this may have a somewhat important bearing on soil fertility work. Based on a determination of potassium by the ashing procedure, the amount of potassium removed from soil by crops may be somewhat larger than has been evident in the past. Attention is directed to the need for further study of the method for the determination of potassium in plant material as a part of the study of the ash method for utilization as a basis for the

determination of mineral elements. Furthermore, consideration might be given to the development of a standardized ash method for all plant (and perhaps animal) material. In *Methods of Analysis* a temperature of about 550°C. is specified for cereal foods, time indefinite. For sugar and sugar products a temperature of about 525°C. to a white ash and constant weight are specified. Based on the above discussion and the data noted, it appears that studies should be made to determine the possibilities and practicality of developing a uniform A.O.A.C. method.

## REPORT ON TOTAL SOLIDS AND ETHER EXTRACT IN FISH

By MANUEL TUBIS (Food and Drug Administration, Philadelphia, Pa.),  
*Associate Referee*

In accordance with the recommendations made in the Associate Referee's report last year (*This Journal*, 25, 710), collaborative study was made of three methods for total solids and three methods for fat on two types of fish.

The samples, consisting of fresh herring and canned salmon preserved with formaldehyde, were prepared by grinding the fish with the preservative and mixing as thoroughly as practically possible. The difficulty of adequately mixing and preparing collaborative samples of a mass containing approximately 75 per cent water and 5 per cent fat, both constituents having a tendency to separate, must be considered in evaluating the results. In order to minimize differences between aliquots and duplicates, the collaborators were requested to allow the samples to come to room temperature, to shake the jars so as to contact the lid and sides in order to incorporate any separated fat and liquid, and then to thoroughly mix the contents before removing a portion. It was also suggested that all subdivisions be weighed as nearly as possible at the same time.

The manner of preparing the dishes and sample was to be similar to that described in the Associate Referee's report of 1940 (*This Journal*, 24, 706), and they were then to be dried according to the following methods:

*Method 1.*—Dry the samples in a vacuum oven at less than 25 mm. pressure to constant weight (ca. 5 hours). Admit dry air into the oven to bring to atmospheric pressure, immediately replace the lid tightly, cool in a desiccator, and weigh soon after room temperature is attained. Reheat for 1 hour and reweigh. Report both results as total solids.

*Method 2.*—Dry the samples at 70° ( $\pm 5^\circ$ ) for 1.5 hours and then at 130°C. ( $\pm 5^\circ$ ) for 1 hour, cool in desiccator, and weigh. This is the method of Veshchezerov.<sup>1</sup>

<sup>1</sup> *Rybnoe Khoz* (U.S.S.R.), No. 3, 29-32 (1938); *Khim. Referat. Zhur.*, I, No. 10 95-96 (1938); C. A., 33, 7913 (1939).

*Method 3.*—Dry the samples in a tight vacuum desiccator over freshly boiled  $H_2SO_4$  (*Methods of Analysis, A.O.A.C.*, 1940, 354, 5) to a minimum weight, weighing the dishes and changing the acid every 24 hours. Use the minimum weight to calculate the total solids. If the samples begin to gain weight, use the minimum weight. Report results, giving duplicate and average figures.

Remove the dried samples for subsequent fat determinations.

The Associate Referee also determined total solids, i.e., moisture, by the Kaye-Leibner-Connor method,<sup>2</sup> but owing to the general unavailability of the apparatus required did not request collaborators to use this method. The results are reported herewith for comparative purposes.

TABLE 1.—Comparative results on total solids by four methods (per cent)

COLLABORATOR*	METHOD 1 VACUUM OVEN		METHOD 2 VESHCHEZEROV		METHOD 3 VACUUM DESICCATOR		KAYE-LEIBNER- CONNOR METHOD
Sample 1, Herring							
A. Alter	26.03		25.54		26.39		
	25.64	25.84	26.02	25.78	26.69	26.54	
G. Kirsten	25.87		26.17		25.58		
	25.87	25.87	25.77	25.97	25.81	25.70	
J. Lemon	27.47		27.70		28.50		
	27.87	27.67	27.50	27.60	28.54	28.52	
H. Shuman	26.45		26.29		26.05		
	26.12	26.29	25.54	25.92	26.28	26.17	
M. Tubis	25.05		25.22		25.88		
	25.20	25.13	25.22	25.22	26.18	26.03	21.98
Mean		26.16		26.10		26.59	
Max. dev. from mean		1.51		1.50		1.90	
Sample 2, Canned Salmon							
A. Alter	26.11		26.06		26.58		
	25.91	26.01	26.10	26.08	26.82	26.70	
G. Kirsten	26.27		26.18		26.45		
	26.29	26.28	26.09	26.14	26.62	26.54	
J. Lemon	26.14		26.19		26.46		
	26.23	26.19	26.09	26.14	26.40	26.43	
H. Shuman	26.09		26.05		26.62		
	26.08	26.09	26.28	26.17	26.60	26.61	
M. Tubis	26.09		25.91		26.73		21.61
	26.06	26.08	25.94	25.93	26.73	26.73	19.86†
Mean		26.13		26.09		26.60	
Max. dev. from mean		0.14		0.16		0.17	

\* All collaborators are members of the Food and Drug Adm., except J. Lemon of the U. S. Department of Interior Technological Lab., College Park, Md.

† Different subdivision at a later date.

TABLE 2.—*Comparison of means for three methods of drying*

HERRING		CANNED SALMON	
METHOD	TOTAL SOLIDS	METHOD	TOTAL SOLIDS
	<i>per cent</i>		<i>per cent</i>
Vacuum oven	26.16	Vacuum oven	26.13
Veshchezerov	26.10	Veshchezerov	26.09
Vacuum desiccator	26.59	Vacuum desiccator	26.60

## DISCUSSION

In Method 1, drying in a vacuum oven, the results reported by the collaborators confirm the work of the Associate Referee, namely, that reheating for 1 additional hour caused a maximum loss of about 1 part in 300, and an average loss of 1 part in 1000 in the case of herring and somewhat less in the case of salmon. Therefore, the 1 hour period of reheating seems to be sufficient to obtain constant weight. The reported figures are given in Table 1. For approximate results, 5 hours' heating should be sufficient in most cases.

In the Veshchezerov method, the first period of drying specified a temperature of 70°C. for 1–5 hours, but later collaborators were requested to make this period 1.5 hours. Although three collaborators had already heated their samples for various periods, the results of each collaborator agree fairly well with those by the vacuum oven method, and the over-all averages are in good agreement with those by the latter method, showing that this period must be at least 1.5 hours but that a longer period is not detrimental. As was formerly noted, the subsequent fat extraction of the fish dried by the Veshchezerov method is somewhat less satisfactory, and therefore this method is less suitable than the vacuum oven method, where fat is to be determined subsequently. It is, however, to be recommended where a rapid method for moisture is desired.

The vacuum desiccator for determining moisture is slow and tedious and no more specific than the other methods, and the subsequent extraction of the fat is not so nearly complete as in the case of either of the above methods, despite the subdivision of the sample. The Associate Referee thinks that the vacuum oven method rather than the vacuum desiccator should be considered the reference method. A comparison of the over-all averages by the three methods is shown in Table 2.

The following methods for the determination of fat were submitted for collaborative study.

*Method 1.*—Weigh ca. 15 gram samples into dry, clean, extraction thimbles. Extract in a continuous extractor for 2 hours, using acetone. Replace the receiving flask, add fresh acetone, and continue to extract for 14 hours. The first flask contains most of the water and fat. Remove the acetone and most of the water by

heating on a steam bath. Place the flasks in a vacuum desiccator over freshly boiled  $H_2SO_4$ , evacuate, and allow to stand overnight, or until most of the water has been removed, as shown by the cessation of foaming and disappearance of the water droplets from the sides of the flasks. Redissolve the fat with ca. 35 ml. of anhydrous ethyl ether, mix well so as to remove any fat held by the gummy material, and filter through a small pledget of cotton or medium sintered-glass funnel into a weighed fat flask or beaker, rinsing both flasks with small portions of the ether and passing this through the funnel until the washings are colorless. Evaporate the ether spontaneously or on a warm surface, finally drying at  $100^{\circ}\text{--}105^{\circ}\text{C.}$  for 1 hour, using a similar flask as a counterpoise. Cool in air and weigh. Reheat for a second hour and reweigh. Again reheat and reweigh and report the fat corresponding to each weighing. See below under discussion for the effects of reheating.

*Method 2.*—Weigh 20 grams of the sample into each of 2 shaking bottles, add 25 grams of anhydrous  $Na_2SO_4$ , and mix intimately, cutting the rod and allowing it to remain in the bottle. Add 100 ml. of anhydrous ethyl ether, stopper with a boiled cork, and shake for 1 hour, using an efficient shaking machine. After shaking, allow any particles to settle and pipet a 20 ml. aliquot through a 7 cm. paper into a beaker weighed with a counterpoise. Wash the paper with five 5 ml. portions of ether. Allow the ether to evaporate spontaneously or remove by gentle warming, and finally dry at  $100^{\circ}\text{C.}$  for 1 hour.

*Method 3.*—Use the following method on the samples dried by the vacuum oven, the Veshchezerov, and the vacuum desiccator methods.

Break up the dried mass in the dishes by means of the rod, roll and quantitatively transfer the foil dish and contents to an extraction thimble, and extract for 16 hours with anhydrous ethyl ether. If the ether is clear, evaporate to dryness, redissolve in ca. 35 ml. of anhydrous ether, and if still perfectly clear again evaporate to dryness and dry as described below. Remove the dried fat by rinsing the flask with successive small portions of the solvent, dry, and weigh, using a similar flask as a counterpoise. Calculate the loss in weight as fat. If the solution is not perfectly clear, filter through a medium-porosity, sintered-glass filter or a small pledget of cotton into a weighed fat flask and evaporate the solvent. Dry the flask containing the fat in an oven at  $100^{\circ}\text{C.}$  for 1 hour, using a similar flask as a counterpoise; cool for 30 minutes and weigh. Again heat for 1 hour, cool, and weigh. If the loss in weight is 1 mg. or less, no further heating is necessary; if the loss in weight is greater than 1 mg. reheat for 1 hour at  $100^{\circ}\text{C.}$ , cool, weigh, and calculate the percentage of fat corresponding to this weight.

## RESULTS

In Table 3 are shown the results obtained by the collaborators on the determination of fat in the two samples by the three methods.

Method 1 is the Stansby-Lemon method with only minor variations. This method was proposed to circumvent the oxidation, polymerization, or other decomposition of the fish oils that occur when they are heated with the fish flesh preparatory to extraction. These authors extract the moisture and fat simultaneously with acetone in the shortest practical time (ca. 16 hours) and purify the crude fat by treatment with ethyl ether. This method was applied to samples of fresh fish, and the results compare very favorably with the A.O.A.C. method for the determination of fat in meat (*Methods of Analysis, A.O.A.C., 1940, 375*). The Associate



TABLE 3.—*Collaborative results on fat (per cent)*

COLLABORATOR	METHOD 1		METHOD 2		VAC. OVEN		METHOD 3 AIR DRIED (135°C.)		VAC. DESICCATOR		KATZ-JENNERT- CONROE METHOD		
A. Alter	5.83		3.73		Sample 1, Herring								
	5.68	5.76	3.50	3.64	5.58	5.09	5.57	5.21	5.57				
	5.91		5.25		5.58	5.33			5.55	5.56			
	5.77	5.84	5.21	5.23	5.31	4.66							
G. Kirsten	5.77		3.51		5.35	4.79	4.91	4.79	6.08				
	5.81				5.57	5.70							
	5.59	5.71	3.57	3.54	5.61	5.72	5.71	5.71	5.79	5.94			
	5.68												
H. Shuman	5.67		3.58		5.45	5.41			5.10				
	5.73	5.70	3.77	3.68	5.38	5.42	5.42	5.42	4.99	5.06			
	5.99		4.60		4.96	5.02			4.70				
	6.10	6.05	4.87	4.74	4.91	5.05	5.04	5.04	5.09	4.89	5.49		
Mean		5.81		4.17	5.38	5.23			5.34				
			4.99*										
			4.96*	4.98									
Sample 2, Canned Salmon													
A. Alter	3.57		3.55		3.74	3.51			3.51				
	4.95	4.26	3.45	3.50	3.80	3.55	3.53		3.13	3.32			
	3.68		3.64		3.55	3.40			3.25				
		3.68	3.57	3.61	3.56	3.43	3.42		3.10	3.18			
G. Kirsten	4.19		3.10		3.37	3.23			3.55				
	4.35		2.99	3.06	3.41	3.39	3.23		3.56	3.56			
	4.25												
	4.33												
H. Shuman	4.16	4.28	3.29		3.47	3.20			3.30				
	4.05		3.26	3.28	3.50	3.28	3.24		3.19	3.25			
	4.11												
	4.21	4.11	4.01		3.42	3.45	3.40		3.39			3.92	
M. Tubis	4.21		4.29	4.15	3.42	3.35			3.49	3.44		3.87	
	4.23	4.22											
Mean		4.11		3.52	3.53	3.36			3.35				

\* Centrifuge method.

Referee considers that this method is a reference method for fat. He submitted it to collaborative study and in spite of the difficulty of preparing five similar subdivisions of two samples and analyzing them at different times, the results are in good agreement.

The rapid semiquantitative method is also that of Stansby and Lemon,<sup>3</sup> and although these authors state that this method is not accurate when less than about 10 per cent of fat is present the Associate Referee thinks that because of its simplicity and rapidity it might give acceptable results where a precision method is not required. The results on these two collaborative samples, each containing about 5 per cent fat, confirm the work of the aforementioned authors, as shown by the results in Table 3. The completeness of the fat extraction by this method depends upon the efficiency of the shaking machine, the intimacy of contact of the solvent and the fish, and the state of subdivision of the latter. The alternative method of centrifuging with fresh ether, as suggested by these same workers, was tried on the herring sample by the Associate Referee, and the results are also shown in Table 3. The method could not be adapted for multiple determinations, and while the results are better than those from shaking, it was not submitted to collaborative study.

In Method 3, the Soxhlet extraction of the samples previously dried by one of the three methods for moisture, the average results do not vary much, considering that the temperature range in the moisture determinations is from 20° to 130°C. In the case of both samples, less fat was extracted from the fish dried by the Veshchezerov method than that dried in a vacuum oven, probably owing to the decreased solubility of the fat in ether. The fat was incompletely extracted from the vacuum desiccator samples, probably due to the incompleteness of drying. The difference between the quantity of fat extracted by this method and that extracted by the Stansby-Lemon method is more marked in samples containing percentages in the range of 4–5 per cent. The time required, the manipulations involved, and the low results obtained are evidences that the vacuum desiccator method would not prove to be a satisfactory reference method.

The method utilizing the apparatus of Kaye, Leibner, and Connor<sup>2</sup> was applied, and these results are given in Table 3. This method combines a simultaneous determination of moisture and fat, is as rapid as any other, and requires a minimum of attention and a single sample. It was not submitted for collaborative study because of the general unavailability of the apparatus. The results obtained are between those obtained by the direct acetone extraction and the vacuum oven method. More work is planned with this method.

The results show that attempting to dry the fish fat to constant weight is difficult, if not impossible and results in decomposition. Therefore, some

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<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 341 (1937).

system of drying that would give a reasonably true figure without too much decomposition should be adopted. In the modified Stansby-Lemon method, Method 1 above, the collaborators were instructed to alternately dry and weigh the fat for 1 hour periods and report the percentage corresponding to each weight. The following information was derived from their figures. In the case of the herring, Sample 1, the average variation between the 1st and 2nd weighings was 1 part in 200, or 0.5 per cent, and between the 2nd and 3rd 1 part in 300, or 0.3 per cent. In the case of the canned salmon, Sample 2, the average variation, covering the work of three collaborators, was about 1 part in 150 between the first and second weighings, and about 1 part in 200 between the second and third. Since in samples containing fat in these percentages this variation only amounts to 0.01–0.03 per cent, not more than two reheatings are necessary and in many cases one reheating is sufficient.

In Method 3, the ether extraction of the variously dried samples, the collaborators were instructed to reheat the fat for one hour. The average variation of the percentage of fat between the first and second weights was 1 part in 170 in the vacuum oven method, 1 part in 500 in the Veshchezerov method, and 1 part in 250 in the vacuum desiccator method. This second reheating should be retained.

#### SUMMARY

The collaborative results on one sample of herring and one sample of canned salmon by three methods for determining total solids and three methods for ether extract (fat) are presented.

#### ACKNOWLEDGMENT

The Associate Referee expresses his gratitude to the collaborators for their generous efforts.

#### RECOMMENDATIONS

It is recommended—

- (1) That the vacuum oven method for the determination of moisture in fish be made tentative.
- (2) That the Veshchezerov method for the rapid determination of moisture be made tentative.
- (3) That the Stansby-Lemon method for the determination of fat be made tentative.
- (4) That the rapid semiquantitative method of Stansby and Lemon be studied collaboratively.
- (5) That the Kaye-Leibner-Connor method for the determination of moisture and fat be studied collaboratively.

## REPORT ON DETECTION OF CARAMEL IN VINEGAR

By A. M. HENRY, *Associate Referee*, and J. W. SANDERS, JR. (U. S. Food and Drug Administration, Atlanta, Ga.)

Lichthardt<sup>1</sup> describes a method for the qualitative detection of caramel in vanilla extracts and liquors that utilizes a dilute solution of tannic and sulfuric acids. This reagent was also used to a lesser extent to detect caramel in vinegar.

Cook and Miller (*This Journal*, 22, 588) made a more thorough investigation of this method, somewhat modified, in its application to the detection of caramel in vinegar. Their results show the general reliability of the method when carefully controlled, but indicate that the method is not sensitive enough for some concentrations of caramel in vinegar and may give positive results on old vinegar that evidently does not contain caramel.

Gulick (*This Journal*, 24, 691) extended this investigation, using numerous samples of commercial vinegars and solutions of caramel of different manufacture and concentrations. He also modified the original Lichthardt reagent by the addition of a small quantity of formaldehyde and performed the test at a different temperature. The results obtained indicate that the sensitivity and reliability are much increased. He proposed a modified procedure for the detection of caramel in vinegar.

This study was continued on the proposed modified procedure. Samples of commercial vinegar and solutions of various samples of caramel (including one prepared in the laboratory from sugar and ammonium carbonate) of different concentrations were studied and compared with the A.O.A.C. methods and the original Lichthardt method. The results confirm the work of Gulick. Dubois<sup>2</sup> made a thorough investigation of the Fuller's earth test for caramel in vinegar, using 16 samples of pure apple cider vinegar and 9 samples of Fuller's earth, and concluded that the method was unreliable, and at best could be used only as a preliminary test.

## COLLABORATIVE STUDY

The Association recommended that the method for the detection of caramel in vinegar be studied collaboratively. Consequently, it was decided to submit samples for a collaborative study of the proposed Lichthardt method, the color removal by Fuller's earth, and the Woodman-Newhall modification of the Amthor test for caramel.

The collaborators were requested to test each of five submitted samples of vinegar, a 4 per cent acetic acid solution and a 4 per cent acetic acid

<sup>1</sup> *J. Ind. Eng. Chem.*, 2, 389 (1910).

<sup>2</sup> U. S. Bur. Chemistry Bull. No. 105, p. 23 (1907).

solution colored with caramel to approximate the color of cider vinegar, by each of the three following methods:

I. *Methods of Analysis*, A.O.A.C., 1940, 16(f), 252.

II. *Ibid.*, 76, p. 481.

III. *Modified Lichthardt Method*:

(a) *Stock solution*.—Dissolve 9.8 grams of tannic acid in ca. 300 ml. of water, add 4 ml. of conc.  $H_2SO_4$ , and dilute to 490 ml. Allow to stand overnight before use. (This solution keeps for a considerable time.)

(b) *Modified Lichthardt reagent*.—Prepare reagent by adding 4 ml. of formaldehyde solution to 96 ml. of stock solution (a). Mix well and filter. Prepare fresh reagent for each day's use.

#### DETERMINATION (QUALITATIVE)

Mix 5 ml. of sample of vinegar and 5 ml. of reagent (b) in test tube and immerse in bath of boiling water for 4 minutes. Remove, cover, and allow to stand overnight. (A brown precipitate settled to bottom of the tube indicates a positive test for caramel. A gray precipitate should be disregarded.)

The vinegars were:

Sample A: Apple cider vinegar.

Sample B: Commercially distilled vinegar with added caramel.

Sample C: Apple cider vinegar (A) to which caramel had been added in the laboratory by the Associate Referee.

Sample D: 75% of apple cider vinegar (A) and 25% of distilled vinegar with caramel (B).

Sample E: Corn sugar vinegar.

Sample F: 4% acetic acid solution prepared by each collaborator.

Sample G: Sample F colored with collaborator's caramel to approximate the color of cider vinegar.

In addition, the collaborators were requested to report all results as positive or negative.

The Associate Referee appreciates the cooperation of the following collaborators in this work:

T. A. Balthis, Dept. of Agriculture and Immigration, Richmond, Va.

H. P. Bennett, U. S. Food and Drug Administration, Kansas City, Mo.

R. H. Dick, U. S. Food and Drug Administration, San Francisco, Calif.

L. W. Ferris, U. S. Food and Drug Administration, Buffalo, N. Y.

S. D. Fine, U. S. Food and Drug Administration, St. Louis, Mo.

K. A. Freeman, Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.

E. M. Hodnett and V. E. Stewart, Florida Dept. of Agriculture, Tallahassee, Fla.

D. A. Holaday, U. S. Food and Drug Administration, San Francisco, Calif.

G. Q. Lipscomb, U. S. Food and Drug Administration, Baltimore, Md.

J. H. Loughrey, U. S. Food and Drug Administration, Boston, Mass.

W. J. Marsh, Dept. of Agriculture & Industries, Montgomery, Ala.

J. A. Mathews, Food Division, U. S. Food and Drug Administration, Washington, D. C.

F. J. McNall, U. S. Food and Drug Administration, Cincinnati, Ohio.

W. H. Naylor, U. S. Food and Drug Administration, Seattle, Wash.

H. Price, State Chemist Laboratory, Vermillion, S. D.

L. B. Rhodes, North Carolina Dept. of Agriculture, Raleigh, N. C.  
J. W. Sanders, Jr., U. S. Food and Drug Administration, Atlanta, Ga.  
Harry Shuman, U. S. Food and Drug Administration, Philadelphia, Pa.  
J. A. Thomas, U. S. Food and Drug Administration, New Orleans, La.

The collaborative results are given in Table 1.

### COMMENTS OF COLLABORATORS

F. J. McNall mentions the unreliability of Method I, but states that Method II furnishes a good sorting-out procedure.

J. H. Loughrey does not believe Method I to be very sensitive or accurate.

K. A. Freeman makes the following comment: "Of the three methods, the modified Lichthardt method seems to be easiest and quickest, and to give more positive results. However, I found it was sometimes difficult to determine whether a precipitate was gray or brown."

D. A. Holaday states that Method I is useless for detection of caramel in vinegar and that the modified Lichthardt method is best.

H. P. Bennett finds the modified Lichthardt to be the most satisfactory, less involved, and to give more clear-cut results.

T. A. Balthis recommends the modified Lichthardt method highly because of its simplicity and states that it is certainly an excellent negative test.

E. M. Hodnett and V. E. Stewart state: *Method I*: "We suggest that 16(f), page 252, A.O.A.C., be more explicit: (a) Specify how far to concentrate the 10% acetic acid solution used in dissolving the precipitate and give the amount of solution left after evaporation, (b) give the procedure for neutralizing the resulting acetic acid solution. A suitable indicator should be specified in order to obtain a definite pH value at neutralization." *Method II*: "We used three different grades of Fuller's earth, two grades from Attapulugus, Georgia, and one grade from an unknown source. No appreciable decolorization was obtained with any of these materials." *Method III*: "We suggest simplifying the preparation of the Lichthardt reagents by using 10 grams of tannic acid and diluting to 500 ml."

J. A. Mathews did not obtain clear-cut results by Method III, but remedied this difficulty by shaking the mixed reagents with HCl-treated Fuller's earth and filtering. He also suggests centrifuging the sample instead of allowing it to stand overnight.

### DISCUSSION OF RESULTS

In general, the results reported for the paraldehyde and phenylhydrazine hydrochloride tests (Method I) are very erratic, and many of the collaborators express their dissatisfaction with the method. Some results were positive on one aliquot and negative on the other, while others were doubtful. Some collaborators obtained positive or doubtful results with 4 per cent acetic acid, while others obtained negative or doubtful results with 4 per cent acetic acid containing caramel. Thus, this method appears to be very unreliable as a criterion for the presence or absence of caramel in vinegar.

The results reported for the Fuller's earth method (Method II) are in closer agreement than are those for Method I. However, there is a wide variation in the percentages of color removed in the same sample by differ-

TABLE 1.—Collaborative results

COLLABORATOR:	SAMPLE A						SAMPLE B						SAMPLE C						SAMPLE D						SAMPLE E						SAMPLE F						SAMPLE G																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
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I. Modified Anthon method

a. Paraldehyde

b. Phenylhydrazine-hydrochloride

II. Fullers' earth method

a. Per cent color removed

b. Conclusion

III. Modified Lighthardt method

ent samples of Fuller's earth. Several collaborators gave the per cent color removed without evaluating as positive or negative. A few samples of Fuller's earth removed less than 50 per cent of the caramel color from colored vinegar, while others removed more than 50 per cent of the color from pure apple cider vinegar. If Fuller's earth were standardized as to the removal of caramel from vinegar, the method might be more reliable, but the indiscriminate selection of this material seems to give erratic and unreliable results.

The results reported for the modified Lichthardt method (Method III) by different collaborators are in much better agreement than are those by either of the other methods. However, a few discrepancies are noted. For example, Collaborator 11 reported caramel in Sample A (pure apple cider vinegar) by this method as well as by each of the other methods. The writers can not account for these results. Also, Collaborator 16 obtained negative results on Sample G, a 4 per cent acetic acid solution to which the collaborator had added his own sample of caramel. In order to account for this result, the writers obtained a sample of this caramel and confirmed these results. However, this caramel differs from most other caramels in being basic instead of acidic. If a very small quantity of sulfuric acid is added to the acetic acid solution colored with this caramel, a positive test is obtained by this modified Lichthardt method. However, such an addition of acid is not advisable, as the acid concentration is somewhat critical, and a large quantity will give positive results even when the vinegar does not contain any caramel.

A survey of the results obtained on Sample B, a commercial distilled vinegar with added caramel (approximately 50 per cent of the color intensity of most apple cider vinegars) and Sample D, 75 per cent Sample A and 25 per cent Sample B, gives an indication of the sensitivity of the test. The writers obtained positive results on all commercially caramel-colored vinegars tested. Also, when Samples A and B were mixed in equal proportions, a positive test was obtained, even though negative results were obtained on Sample D.

This collaborative study indicates the general reliability of the modified Lichthardt method as a means of detecting caramel in vinegar, and especially its value as a negative test. It seems to be superior to either of the other two methods in simplicity, reliability, time required, and ease of manipulation.

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## REPORT ON SPECTROPHOTOMETRIC METHODS

QUANTITATIVE DETERMINATION OF QUININE BY  
ABSORPTION SPECTROPHOTOMETRY

By J. CAROL (U. S. Food and Drug Administration, Chicago, Ill.),  
*Associate Referee*

A method for the determination of quinine by ultraviolet spectrophotometry was described in a previous paper (*This Journal*, 25, 524). It was there shown that quinine can be determined in the presence of many other compounds by its absorption at approximately 340  $m\mu$ . In this region quinine was found to follow the Beers-Lamberts law closely enough to enable accurate determinations to be made when a standard curve containing points at 0.5, 1.0, and 1.5 mg. of quinine per 100 ml. is prepared at the time of analysis.

The original work was done with a spectrophotometer having a very limited range in the ultraviolet and a fixed slit that isolated a portion of the spectrum 35  $m\mu$  wide. The writer has now available the precise quartz spectrophotometer described under "Experimental." With this instrument a complete ultraviolet absorption curve of quinine in dilute hydrochloric acid has been made, and its applicability in making quantitative measurements in each absorption region has been investigated.

## EXPERIMENTAL

A Beckmann quartz spectrophotometer having a spectrum range of 200–2000  $m\mu$  and an isolated spectrum band of 2  $m\mu$  or less (over all but extreme ends of spectrum) was used. Matched 1 cm. quartz cells were used for all measurements. Calibration was effected by checking the wave lengths of the principal peak absorptions for benzene.

Quinine NF was recrystallized twice from benzene and dried to constant weight at 110°C. M.P. 174–174.5°C. (Corr.).

The spectral absorption curve in Figure 1 was drawn by making absorption ( $E = -\text{Log transmittance}$ ) measurements from 220 to 400  $m\mu$  of quinine in 0.1 *N* hydrochloric acid and by using a blank 0.1 *N* hydrochloric acid. Table 1 shows the location and magnitude of the three regions of maximum absorption.

TABLE 1

$m\mu$	$E_{1\text{cm.}}^{1\%}$
250.5	901
318.0	142
347.5	168

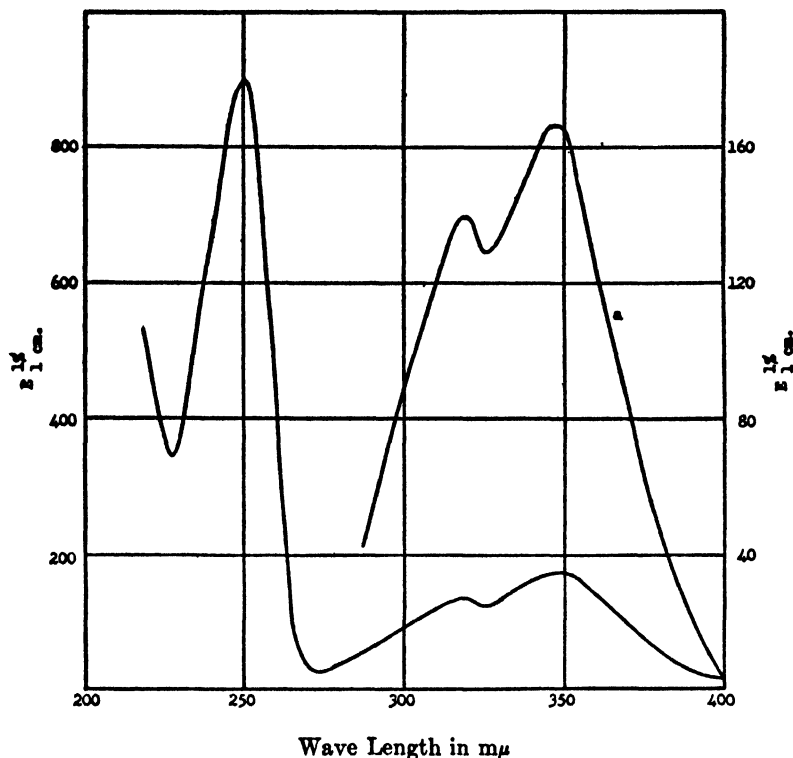


FIG. 1.—The absorption spectra of quinine in 0.1 *N* HCl. (a) is a magnified portion of the curve from 280–400  $m\mu$  and should be read on the right hand ordinate.

A series of solutions containing 0.2–5.0 mg. of quinine (anhydrous) per 100 ml. of 0.1 *N* hydrochloric acid was prepared, and the absorptions of

TABLE 2

QUININE ANHYDROUS/ 100 ML. 0.1 <i>N</i> HCl	E 1 CM. 250.5 $m\mu$	E 1 CM. 318.0 $m\mu$	E 1 CM. 347.5 $m\mu$
mg.			
0.2	0.181	—	—
0.5	0.452	—	—
1.0	0.901	0.142	0.168
2.0	—	0.278	0.338
3.0	—	0.420	0.502
4.0	—	0.560	0.672
5.0	—	0.701	0.840

the solutions (E) relative to a blank of 0.1 *N* hydrochloric acid were measured at 250.5, 318.0, and 347.5  $m\mu$ . These data are given in Table 2.

## DISCUSSION

The Beers-Lamberts law is followed at each maxima\* as shown by the straight lines produced in Figure 2, where E is plotted against concentration. Consequently quantitative measurements could be made at 250.5, 318.0, and 347.5  $m\mu$ . Determinations at 347.5  $m\mu$  are most useful for drug analysis because practically all alkaloids and synthetics exhibit no absorption in this region, while many absorb strongly at 250.5  $m\mu$ . This property allows quinine to be determined directly at 347.5  $m\mu$  without separation.

A number of substances, especially yellow compounds, absorb strongly in the region of 350  $m\mu$ . It is possible that quinine may be determined in their presence by measurements at 250.5  $m\mu$ . To do this it would be necessary to establish that the interfering compounds have no absorption at 250.5  $m\mu$ .

The following modified method for quinine eliminates the preparation of a standard curve and requires but two E determinations, one of the sample and one of the standard solution.

## METHOD

## APPARATUS

A spectrophotometer suitable for measuring absorption in the ultraviolet. A slit that isolates a portion of the spectrum 5  $m\mu$  or less is desirable. Two matched 1 cm. absorption cells (quartz cells are required if measurements at 250.5  $m\mu$  are made).

## REAGENTS

*Hydrochloric acid*.—0.1 N. Normality need only be approximate.

*Quinine anhydrous*.—Recrystallize quinine NF twice from benzene. Dry to constant weight at 110°C.

*Phosphoric acid*.—85% sirupy  $H_3PO_4$ .

## STANDARD SOLUTION

Prepare a standard containing 5.0 mg. of anhydrous quinine per 100 ml. of 0.1 N HCl. (This solution keeps indefinitely.)

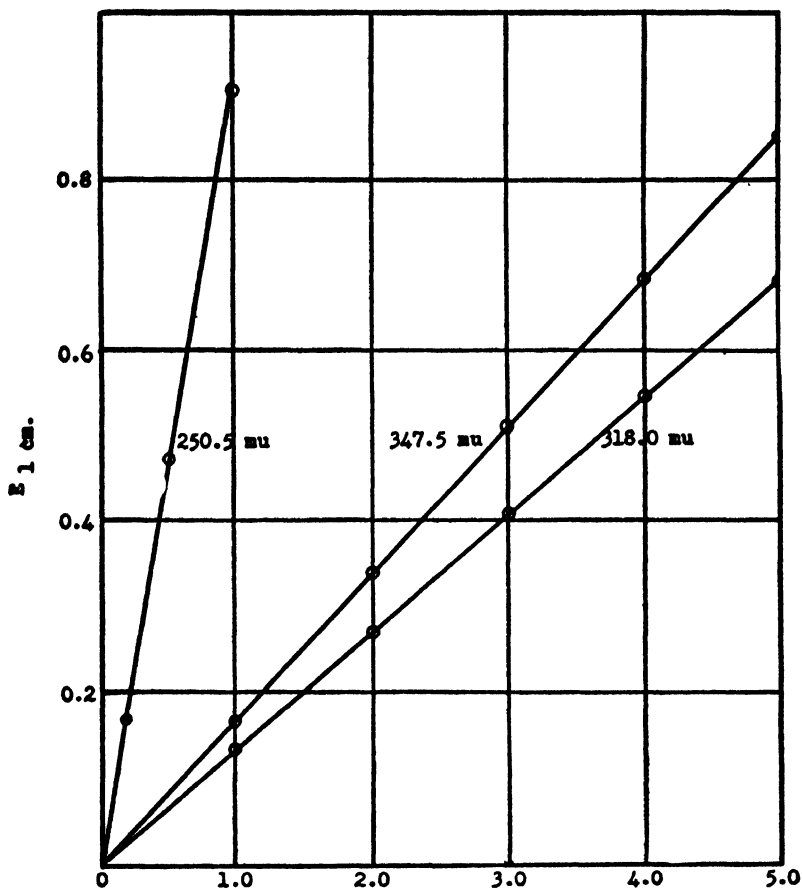
## DETERMINATION

I. Applicable in presence of those compounds having no absorption in HCl solution at 347.5  $m\mu$ . These compounds include strychnine, atropine, ephedrine, caffeine, acetylsalicylic acid, acetanilid, acetophenetidin, camphor, phenolphthalein, glycerol, alcohols; most green, blue, and red dyes; and sugars, all of which are frequently found in preparations containing quinine.

Accurately weigh or measure a quantity of the sample containing ca. 0.1 gram of quinine and transfer to a 1000 ml. volumetric flask. Dissolve in 25 ml. of HCl (1+4) and make to volume so as to have the final concentration ca. 0.1 N in HCl. Filter solution if not perfectly clear. Pipet an aliquot containing 2–5 mg. of quinine

\* In the previous paper quinine solutions deviated from the Beers-Lamberts law. This deviation was probably due to instrumental error. T. R. Hogness, F. P. Zscheile, Jr., and A. E. Sidwell, Jr.<sup>1</sup> state that scattered radiation, uncorrected dark current, width of spectrum band isolated, and length of absorption cells have an effect on the determination of the absorption coefficient. Errors due to these causes increase with increases in concentration producing apparent deviation from the Beers-Lamberts Law.

<sup>1</sup> *J. Phys. Chem.*, 41, 379 (1937).



Mg. Quinine Anhydrous per 100 ml. 0.1N HCl  
 FIG. 2.—Plot of E against concentration at wave length of 3 maxima.

into a 100 ml. volumetric flask and fill to mark with 0.1 N HCl. Determine the absorption (E) relative to a blank of 0.1 N HCl at 347.5 mμ. Determine the absorption (E) of the standard solution relative to a blank of 0.1 N HCl at 347.5 mμ.

$$\text{mg. quinine (anh.) in aliquot} = \frac{E_{\text{sample}}}{E_{\text{standard}}} \times 5.0.$$

II.—Applicable in presence of ferric compounds, such as elixir of iron, quinine, and strychnine.

Proceed as directed in I, but add 10 ml. of the sirupy  $\text{H}_2\text{PO}_4$  to the solution in a 100 ml. flask before making to volume. The standard solution should contain 10 ml of sirupy  $\text{H}_2\text{PO}_4$  per 100 ml. and the blanks should consist of 0.1 N HCl containing 10 ml. of sirupy  $\text{H}_2\text{PO}_4$  per 100 ml.

III.—Applicable in presence of interfering substances. Before determining quinine by its absorption at 347.5 mμ, separate it from the following compounds: Aloin, podophyllin, anthraquinone derivatives, other cinchona alkaloids, and yellow dyes because they absorb light in the region of 347.5 mμ.

## REPORT ON METHYLENE BLUE IN MIXTURES

By H. O. MORAW (U. S. Food and Drug Laboratory, Chicago, Ill.),  
*Associate Referee*

Investigational work was conducted in 1942 on authentic mixtures simulating those commonly dispensed as follows:

1. In gelatine capsules—methylene blue, and oils of copaiba, santal, and wintergreen.

2. Same as (1) plus salol.

3. In coated tablets—methylene blue, oils of santal, nutmeg, cassia, copaiba; starch, calcium carbonate, talc; lactose, etc.

The U.S.P. perchlorate (gravimetric) method of determination was employed after separation by the A.O.A.C. procedure, except that after the latter was found inapplicable for Mixture 3 two modifications were tried. The following results were obtained:

MIXTURES 1 AND 2	MIXTURE 3
<i>per cent</i>	<i>per cent</i>
101.5	183.0
101.4	103.7 <sup>a</sup>
101.0	86.1 <sup>b</sup>
101.3	77.1 <sup>b</sup>

<sup>a</sup> Instead of A.O.A.C. method, the water extract was filtered through a Gooch crucible to remove insoluble material.

<sup>b</sup> Sample was shaken with water several hours in volumetric flask. Made to volume, allowed to stand 14 hours or longer, decanted through Gooch crucible without suction.

Results on Mixtures 1 and 2 are about as satisfactory as could be expected for this type of product. Those on Mixture 3 were unsatisfactory by the present A.O.A.C. procedure of separation, or as modified according to footnotes (a) and (b) above. Filtration (a) was too slow to be practical. Modification (b), called to the attention of the Associate Referee by L. E. Warren, apparently did not effect complete solution or separation of the methylene blue from the coated tablet mixtures as prepared.

It is recommended that the subject be studied further.

## REPORT ON ALIZARIN IN MADDER LAKES

By W. C. BAINBRIDGE (H. Kohnstamm & Company, Inc.,  
Brooklyn, N. Y.), *Associate Referee*

The alizarin content of three madder lakes was analyzed collaboratively, according to the method of the Associate Referee (*This Journal*, 25, 956).

Table 1 lists the findings of five collaborators in the order the results were received.

TABLE 1.—*Collaborative results on alizarin*

ANALYST NO.	SAMPLE 1	SAMPLE 2	SAMPLE 3
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	41.6	30.6	18.1
	41.0	31.6	18.2
	41.6	30.2	18.4
		31.7	
2	40.7	30.2	17.95
	40.35	30.1	17.4
	41.35	29.45	17.1
	40.7	30.3	17.2
3	41.6	31.7	20.6
	39.6	30.4	19.7
	40.8	31.0	18.5
	40.6	30.2	17.7
	40.7	31.1	19.4
4	38.61	28.78	16.06
5	39.0	31.25	19.5

The Associate Referee is indebted to the following collaborators whose cooperation made this report feasible:

Kenneth A. Freeman, Food and Drug Adm., Washington, D. C.

I. Hanig, H. Kohnstamm & Co., Inc., Brooklyn, N. Y.

Wm. H. King, Food and Drug Adm., New Orleans, La.

Milton Manes, Food and Drug Adm., Washington, D. C.

T. A. Martone, E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.

These reports show that a deviation as great as 3 per cent may occur in the analysis of a single sample by the same man, while the deviation on the same sample by two men may be as great as 4 per cent. This difference is undoubtedly due to the small size of the sample that is being analyzed. However, larger samples would be unmanageable because of the low solubility of alizarin in ether.

#### COMMENTS OF COLLABORATORS ON ALIZARIN IN MADDER LAKES

*I. Hanig.*—It is suggested that a large sized sample, possibly 0.5 gram, may cut down on errors in weighing and achieve closer checks.

*Milton Manes.*—It may also be mentioned that the first HCl extracts of the samples usually stood several hours or overnight before extraction with ether. In extracting with the NaOH reagent, it was not always possible to remove the last traces of blue color from the ether layer, and a small amount of blue material was usually found separated at the interface between the alkali and ether layers.

In re-extracting alizarin from the acidified NaOH solution, it was found that

four successive extractions with 25 ml. portions of ether extracted all the color from the acid layer.

#### SUMMARY AND RECOMMENDATIONS

The collaborative study of the determination of alizarin in madder lakes has resulted in findings that are relatively satisfactory. It is recommended therefore that the method be adopted as tentative.

#### CORRECTION

In *This Journal*, 26, 145 (1943), line 5, change "higher" to read "lower."

## CONTRIBUTED PAPERS

### SYSTEMATIC GROUP SEPARATION OF MIXTURES OF FD&C, D&C, AND EXT. D&C COLORS BY USE OF IMMISCIBLE SOLVENTS

By LOUIS KOCH (H. Kohnstamm & Co., Inc., Brooklyn, N. Y.)

Color analysis, based on the use of immiscible solvents, was studied comprehensively by Mathewson (1, 2). Chromatographic procedures were investigated by Ruggli and Jensen (3, 4), who reported considerable success in the separation of dye mixtures into their individual components. Others (5, 6, 7, 8, 9, 10, 11) have attacked this problem by methods that lend themselves less readily to the systematic isolation of a limited number of colors.

The proposed scheme is not infallible, but it has been used advantageously for the past two years in the laboratories of H. Kohnstamm & Co. No efforts have been made as yet to separate a mixture of more than a few dyes of one group into its component parts because of the difficulty involved with groups that contain as many as nineteen colors. It is the opinion of the writer, however, that a combination of fractional separation by means of immiscible solvents and chromatographic analysis may ultimately overcome this obstacle.

#### PROCEDURE

Dissolve 5–15 mg. of the mixture in 24 ml. of water, and make alkaline with 6 ml. of 10% NaOH. If the unknown is insoluble in water, boil a similar sized sample with 30 ml. of a 2% NaOH solution for 1 minute, cool, and filter.

Extract the solution or filtrate with 30 ml. of ether, repeating the extraction with a second and a third portion of ether, if necessary. Wash the extracts with 20 ml. volumes of 0.5% NaOH solution, passing the washings through the separatory funnels in the original sequence. Discard the washings if they are lightly tinted, but reserve them if they are deeply colored (cf. "Washings" below).

Acidify the color solution, from which the alkaline ether extracts have now been removed, with 10 ml. of acetic acid, and extract with one or more 30 ml. portions of ether. Wash the extracts with 20 ml. volumes of a 0.5% acetic acid solution, discarding or reserving the washings according to the intensity of the color. The ether extracts contain:—

*Group 1.*—Alkaline ether extract: Rhodamine B (D&C Red Nos. 19, 20, 37).

*Group 2.*—Acid ether extract: Erythrosine (FD&C Red No. 3, D&C Red No. 3), Fluorescein (D&C Yellow Nos. 7, 8, 9), Tetrabromofluorescein (D&C Red Nos. 21, 22, 23), Tetrachlorofluorescein (D&C Red Nos. 24, 25, 26), Tetrachlorotetrabromofluorescein (D&C Red. Nos. 27, 28), Bluish Orange T.R. (D&C Red No. 29), Dibromofluorescein (D&C Orange Nos. 5, 6, 7), Dichlorofluorescein (D&C Orange Nos. 8, 9), Diiodofluorescein (D&C Orange Nos. 10, 11, 12, 13), Orange T.R. (D&C Orange No. 14), Alizarin (D&C Orange No. 15), Dibromodiiodofluorescein (D&C Orange No. 16), Dichlorotetraiodofluorescein (Ext. D&C Red Nos. 4, 5, 6).

Dilute the color solution, from which groups 1 and 2 have been extracted, to 60



ml. with a 20% salt solution. Extract with 30 ml. portions of amyl alcohol. Wash the amyl alcohol extracts with a 5% salt solution, reserving or discarding the washings as usual. The amyl alcohol extract contains:—

*Group 3.*—Orange 1 (FD&C Orange No. 1, D&C Orange No. 1), Guinea Green B (FD&C Green No. 1, D&C Green No. 1), Quinoline Yellow W.S. (D&C Yellow No. 10), Orange II (D&C Orange No. 4), Lithol Rubin B (D&C Red Nos. 6, 7), Deep Maroon (D&C Red No. 34), Resorcin Brown (D&C Brown No. 1), Alizarin Cyanine Green F (D&C Green No. 5), Alizarin Astrol B (D&C Blue No. 5), Patent Blue (D&C Blue Nos. 7, 8), Acid Violet 6B (D&C Violet No. 1), Metanil Yellow (Ext. D&C Yellow Nos. 1, 2), Methylene Blue (Ext. D&C Blue Nos. 1, 2), Violamine R (Ext. D&C Red No. 3), Alizarin Carmine (Ext. D&C Red No. 7), Coomassie Black (Ext. D&C Black No. 1), Anthraquinone Violet (Ext. D&C Violet No. 1), Alizuril Purple (Ext. D&C Violet No. 2), Fast Light Yellow (Ext. D&C Yellow No. 3).

Extract the remaining color solution with 15 ml. portions of a 1+2 CCl<sub>4</sub>-dichlorhydrin mixture. Wash the extracts with a 25% salt solution. Reserve or discard the washings in the usual manner. The extract contains:—

*Group 4.*—Brilliant Blue FCF (FD&C Blue No. 1, D&C Blue No. 1), Light Green SF Yellowish (FD&C Green No. 2, D&C Green Nos. 2, 4), Fast Green FCF (FD&C Green No. 3, D&C Green No. 3), Alphazurine FG (D&C Blue No. 4).

Acidify the color solution, from which groups 1, 2, 3, and 4 have now been removed, with 1.4 ml. of HCl for every 40 ml. of solution, and extract with one or more 30 ml. volumes of amyl alcohol. Wash the extracts with 0.125 *N* HCl. Reserve or discard the washings as usual. The amyl alcohol contains:—

*Group 5.*—Naphthol Yellow S (FD&C Yellow No. 1, D&C Yellow No. 1), Ponceau 3R (FD&C Red No. 1, D&C Red No. 1), Ponceau SX (FD&C Red No. 4, D&C Red No. 4), Ponceau 2R (D&C Red No. 5), Naphthol Blue Black (D&C Black No. 1), Croceine Scarlet MOO (Ext. D&C Red No. 13).

Add excess HCl to the remaining dye solution, and extract with 30 ml. portions of amyl alcohol. The residual solution should be colorless. The amyl alcohol contains:—

*Group 6.*—Tartrazine (FD&C Yellow No. 5, D&C Yellow No. 5), Sunset Yellow FCF (FD&C Yellow No. 6, D&C Yellow No. 6), Amaranth (FD&C Red No. 2, D&C Red No. 2), Indigotine (FD&C Blue No. 2, D&C Blue No. 2), Orange G (D&C Orange No. 3), Acid Fuchsin D (D&C Red No. 33), Amidonaphthol Red 6B (Ext. D&C Red No. 1), Pigment Scarlet NA (Ext. D&C Red No. 2), Alizarin Saphirol (Ext. D&C Blue No. 4), Naphthol Green B (Ext. D&C Green No. 1).

If the unknown was insoluble or slightly soluble in water:—

Boil a new sample with 30 ml. of an acid mixture containing 1 part of conc. acetic acid plus 1 part of 1+1 HCl for 30 seconds. Cool, and filter. Add 30 ml. of ether to the filtrate, agitate in the extraction funnel, and then dilute with 30 ml. of water. Agitate again. Extract with 2 more volumes of ether to complete the extraction. Wash the combined ether extracts with *N* HCl. Discard washings. The ether contains:—

*Group 7.*—Lithol Red (D&C Red Nos. 10, 11, 12, 13), Lake Red C (D&C Red Nos. 8, 9), Lake Red D (D&C Red Nos. 14, 15, 16), Brilliant Lake Red R (D&C Red No. 31), Alizarin (D&C Orange No. 15).

Digest a 10–25 mg. sample of the unknown with hot benzol, and filter. The benzol contains:—

*Group 8.*—Yellow AB (FD&C Yellow No. 3), Yellow OB (FD&C Yellow No. 4), Orange SS (FD&C Orange No. 2), Sudan Red II (FD&C Red No. 32), Toney Red (D&C Red No. 17), Oil Red OS (D&C Red No. 18), Helindone Pink CN (D&C

Red No. 30), Alizarin Green SS (D&C Green No. 6), Alizuroil Purple SS (D&C Violet No. 2), Rhodamine B Stearate (D&C Red No. 37), Toluidine Red (D&C Red No. 35), Chlorinated-p-nitraniline Red (D&C Red No. 36), Hexyl Blue (Ext. D&C Blue No. 5), Hansa Yellow (Ext. D&C Yellow No. 5), Hansa Orange (Ext. D&C Orange No. 1), Quinoline Yellow SS (D&C Yellow No. 11).

#### NOTES

*Group 1.*—Rhodamine B is practically decolorized by alkali; it bleeds out of the ether layer with a 0.5 per cent sodium hydroxide solution, and it is washed out of the ether by a 2.5 per cent acetic acid solution, the color being restored. Rhodamine B Stearate is extracted also in Group 8.

*Group 2.*—All the dyes in this group are washed out of the ether layer with a 1+9 ammonia solution. Alizarin is changed to a purple shade by this treatment. Alizarin and Brilliant Lake Red R are extracted also in Group 7. Red Lake D may bleed strongly into Group 2.

*Group 3.*—Quinoline Yellow bleeds into other groups. Lithol Rubin is yellow in alkali and red in acid. Guinea Green B is decolorized by alkali. Metanil yellow is changed to a violet by acid. Methylene Blue turns violet after boiling with a 2 per cent alkali solution, and may bleed into Groups 1 and 2. Red Lake C may bleed into this group, but the dye will not easily wash out of the amyl alcohol with water. Acid Violet 6B is decolorized by alkali. Anthraquinone Violet may bleed into Groups 1 and 2. Patent Blue is not wholly extracted in Group 3, but is completely removed in Group 4. It turns violet on boiling with dilute alkali. Deep Maroon is slightly soluble in a 2 per cent alkali solution. To remove the dyes of Groups 3, 5, and 6 from the amyl alcohol, dilute with petroleum benzin, and wash with water.

*Group 4.*—Brilliant Blue and Alphazurine FG turn a violet-red shade when boiled with a 2 per cent sodium hydroxide solution. Light Green is decolorized by alkali. Fast Green is changed to a purplish blue by alkali. To remove the dyes of Group 4 from the organic solvent, dilute with carbon tetrachloride and wash with water.

*Group 5.*—Naphthol Yellow is nearly decolorized by acid; with sodium hydrosulfite in ammoniacal solution, a rose-red color is produced. Ponceau 2R and Ponceau 3R are easily destroyed by alkaline peroxide whereas Ponceau SX is not. Naphthol Blue Black bleeds into Groups 3 and 4; in Group 4 the 25 per cent salt solution precipitates the dye out of the organic solvent. Croceine Scarlet MOO is dark red in the presence of alkali.

*Group 6.*—Alizarin Saphirol extracts with difficulty.

*Group 7.*—Lithol Red will not wash out of the ether layer with water or with 1+9 ammonia. The ether solution of Red Lake C will bleed slightly when washed with normal hydrochloric acid, and the color will wash out completely with water. Red Lake D and Brilliant Lake Red R will wash out of the ether layer with 1+9 ammonia. Group 2 will bleed into this group and will wash out of the ether layer with 1+9 ammonia. Dyes of

Group 8 may bleed occasionally and may be mistaken for lithol red. Deep Maroon is extracted with ether, washes out very slowly with normal hydrochloric acid and nearly wholly with water, leaving a residual dye that is extractable with 1+9 ammonia.

*Group 8.*—Colors of Group 2 may bleed into the benzol, but are removed with dilute alkali. Helindone Pink has a yellowish fluorescence in benzol solution. Microscopic examination of the crystal structure and the melting point determination will be of great assistance in the identification of the dyes in Group 8.

Groups 2-6 and Group 8 contain dyes of the food-color class, and the procedure of the A.O.A.C. is recommended if the sample is in this category.

*Washings.*—If, in the opinion of the analyst, the washings contain dyes other than those found in the group analysis, add excess HCl and extract with amyl alcohol. Discard the residual washings if they are colorless, but if they are colored add solid salt to bring the salt concentration to 12.5% and extract with portions of CCl<sub>4</sub>-dichlorhydrin mixture. Dilute the amyl alcohol with petroleum benzin and remove the colors with water. Evaporate the water solution to dryness, and repeat the group analysis, 1-6. Dilute the CCl<sub>4</sub>-dichlorhydrin extract with an equal volume of CCl<sub>4</sub>, and remove the coloring matters with water. Evaporate the water solution to dryness, and test for the dyes of Group 4.

#### DISCUSSION

Fortunately, commercial samples rarely contain more than five individual dyes, and the usual number of colors in a mixture lies between two and four. Hence the analysis of an unknown will not be so complex as the number of dyes per group would indicate.

Specific consideration should be given to the possibility of "bleeds" caused by the slight solubility of a dye in an immiscible solvent at a pH slightly higher or slightly lower than the one at which the main portion extracts. The presence of subsidiary and isomeric dyes may also lead to erroneous conclusions. Much will therefore depend upon the familiarity that the analyst establishes with the characteristics of each color.

It would be advantageous to the color chemist to tabulate the color reactions of these dyes with concentrated sulfuric acid, 10 per cent sodium hydroxide if water soluble, and 10 per cent alcoholic potassium hydroxide if water insoluble, so that indicative or confirmatory tests are available. A list of these reactions has not been included by the writer because color descriptions of two individuals very seldom coincide.

If the analyst suspects that there is more than one dye in a given group, it will be found that passing the mixture through a column of bauxite and talc (4+1) will frequently divide the mixture into its individual components.

#### SUMMARY

A method for the analysis of permitted food, drug, and cosmetic dyes, by the use of immiscible solvents, has been outlined.

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PERIODATE REACTION APPLIED TO COSMETIC  
INGREDIENTSDETERMINATION OF GLYCEROL, ETHYLENE GLYCOL,  
PROPYLENE GLYCOL

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Federal Security Agency, Baltimore, Md.)

Malaprade (1, 2, 3) has shown that the periodates have a selective oxidizing action on 1, 2 glycols and certain other  $\alpha$ -hydroxy compounds. Numerous applications of the Malaprade reaction have since been made, both for the study of structure (4-8), and as quantitative methods (9-18) for poly-hydroxy and  $\alpha$ -amino-hydroxy compounds.

Analytical applications of the periodate reactions are based primarily on the determination of the amount of periodate reduced and on determinations of the products of oxidation. Glycerol, ethylene glycol, and propylene glycol are among the compounds that react with cold periodate and are of particular interest in the analysis of cosmetics. Their oxidation products include formic acid, formaldehyde, and acetaldehyde.

Semimicro volumetric methods are described here for the determination of these oxidation products and of reduced periodate. These methods incorporate modifications of several published procedures. Calculations are also included for the estimation of glycerol, ethylene, and propylene glycols, either alone or occurring together.

## METHODS

## REAGENTS

**Potassium periodate.**—0.02 *M*. Dissolve 4.6 grams of  $\text{KIO}_4$  in ca. 500 ml. of hot water. Dilute to ca. 900 ml. with water, cool to room temperature, and make to 1000 ml. (Filter through sintered glass or asbestos if any filter fibers are present in the  $\text{KIO}_4$ .)

**Potassium arsenite.**—0.02 *N*. Dilute 100 ml. of U.S.P. 0.1 *N*  $\text{KAsO}_2$  to 500 ml. with water.

*Iodine*.—0.02 *N*. Dilute 100 ml. of U.S.P. 0.1 *N* iodine to 500 ml. with water.

*Sodium hydroxide*.—0.02 *N*. Dilute 100 ml. of U.S.P. 0.1 *N* NaOH to 500 ml. with water.

*Sodium bisulfite*.—5%. Dissolve 5 grams of reagent NaHSO<sub>3</sub> in 100 ml. of water.

*Borax-sodium carbonate mixture*.—Dissolve 4 grams of Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10 H<sub>2</sub>O and 5 grams of Na<sub>2</sub>CO<sub>3</sub> in 100 ml. of water.

*Aminoacetic acid*.—U.S.P. grade.

*Starch indicator*.—Mix 0.5 gram of soluble starch with 10 ml. of cold water and add 90 ml. of boiling water. Heat to boiling for ca. 5 minutes. Cool before use.

#### OXIDATION WITH PERIODATE

Have the sample solution to be tested neutral to methyl red and contain not more than 45 mg. of glycerol or its equivalent. (Excess periodate must be present in the oxidation mixture.) Make preliminary tests on unknown samples to determine proper sample size. Test for periodate by adding NaHCO<sub>3</sub> and KI to test portions; if excess is present, iodine will be liberated.

Transfer the neutralized sample to a glass-stoppered 100 ml. volumetric flask and add 50 ml. of 0.02 *M* KIO<sub>4</sub>. Make to 100 ml. with water, mix well, and allow to stand ca. 1 hour.

Use aliquots of this oxidized mixture in each of the following determinations:

#### DETERMINATION

(1) *Formic Acid*.—Transfer a 20 ml. aliquot of the oxidized mixture to a titration flask. Add a drop of methyl red indicator solution and titrate with the NaOH to a clear yellow. Reserve the solution for determination of excess periodate.

Apply appropriate corrections for any acidity in the 0.02 *M* KIO<sub>4</sub>.

1 ml. of 0.02 *N* NaOH = 0.92 mg. of formic acid  
= 1.84 mg. of glycerol

(2) *Excess Periodate*.—After the titration with NaOH dilute the solution to ca. 50 ml. with water. Add ca. 0.5 gram of NaHCO<sub>3</sub>, 0.2 gram of KI, and 5 ml. of the starch indicator. Titrate immediately with the KAsO<sub>2</sub> to the disappearance of the blue color. Allow ca. 5 minutes for the reaction to reach equilibrium and to determine the final end point.

Standardize 10 ml. of the KIO<sub>4</sub> by the same titration procedure used with KAsO<sub>2</sub>. 10 ml. of 0.02 *M* KIO<sub>4</sub> = 20 ml. of 0.02 *N* KAsO<sub>2</sub>.

The difference between the two titrations is a measure of the amount of periodate reduced.

1 ml. of 0.02 *N* KAsO<sub>2</sub> = 2.3 mg. of KIO<sub>4</sub>  
= 0.46 mg. of glycerol  
= 0.62 mg. of ethylene glycol  
= 0.76 mg. of propylene glycol

(3) *Total Aldehyde* (Formaldehyde and Acetaldehyde).—Pipet a 20 ml. aliquot of the oxidized mixture into ca. 5 ml. of the NaHSO<sub>3</sub> solution. Let stand for 30 minutes at room temperature. Dilute with water to ca. 50 ml. Add 5 ml. of starch indicator, and sufficient strong (ca. 0.5 *N*) iodine solution to destroy excess sulfite. Discharge the blue color with a drop of the NaHSO<sub>3</sub> solution and make a careful adjustment to the starch end point with the iodine solution.

Add 10 ml. of the borax-Na<sub>2</sub>CO<sub>3</sub> mixture and titrate with the iodine solution to an end point that is stable for 1 minute or longer. The latter titration is a measure of the sulfite bound as aldehyde complexes, i.e., a measure of total aldehydes present.

1 ml. of 0.02 *N* Iodine  
(from total aldehyde)

=0.30 mg. of formaldehyde.

=0.44 mg. of acetaldehyde.

=0.31 mg. of ethylene glycol.

=0.38 mg. of propylene glycol.

=0.46 mg. of glycerol.

(4) *Acetaldehyde*.—

Assemble a gas absorption train, consisting of four 6×1 inch test tubes fitted with 2-hole rubber stoppers, each stopper carrying one short piece of glass tubing and one long enough to reach to the bottom of the test tube. Connect with rubber tubing so that CO<sub>2</sub> gas under pressure will bubble through any liquid in the first tube and then through the liquids in the remaining tubes.

Transfer a 20 ml. aliquot of the oxidized mixture of the sample to Tube 1 and add ca. 2 grams of NaHCO<sub>3</sub>. In Tube 2 place ca. 0.2 gram of aminoacetic acid, 1 gram of NaHCO<sub>3</sub>, and 10 ml. of water. Place ca. 1 ml. of the NaHSO<sub>3</sub> solution and 15 ml. of water in each of Tubes 3 and 4.

Attach a CO<sub>2</sub> supply to Tube 1 and force the gas through the train at the rate of ca. 1½ liters per minute. Continue the aspiration for ca. 1 hour, then transfer the contents of Tubes 3 and 4 to a titration flask. The volume, including wash water, should be ca. 50 ml.

Add starch indicator, destroy excess sulfite with strong iodine, and determine aldehyde bisulfite as directed under "Total Aldehydes."

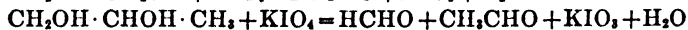
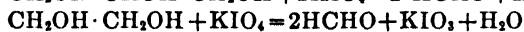
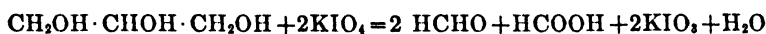
1 ml. of 0.02 *N* Iodine  
(from acetaldehyde)

=0.44 mg. of acetaldehyde.

=0.76 mg. of propylene glycol.

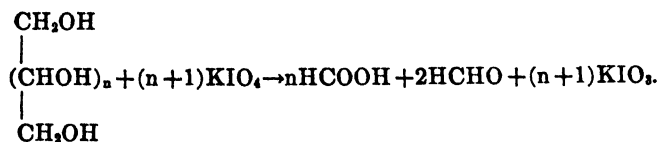
## DISCUSSION

*Periodate Oxidation*.—The reactions of glycerol, ethylene glycol, and propylene glycol with periodate have been shown to correspond to the following equations (3), (13), (18):



It will be noted that each formaldehyde represents a primary carbinol group in the original alcohol or glycol. This has been made the basis for methods to determine such groups in carbohydrates (17).

Formic acid appears to be formed from secondary alcohol groups that are adjacent to primary alcohol groups. In polyhydroxy alcohols such as glycerol, erythritol, and sorbitol the number of mols of formic acid corresponds to the number of secondary alcohol groups (3).



Acetaldehyde would probably be derived from all compounds containing a methyl group adjacent to α-di-hydroxy group. The formation of

acetaldehyde is the basis of methods for the determination of threonine (15) and methyl pentoses (16), as well as propylene glycol (18).

Other oxidation products of the periodate reactions include glyoxylic acid from polyhydroxy acids (9), glycolic acid from hydroxy ketones (9), and ammonia from  $\alpha$ -amino alcohols (12). Methods for these products have not been considered in this report, however.

*Determination of Periodate.*—A periodate salt neutral to methyl red is preferred to periodic acid since the use of such a salt permits the direct titration of formic acid produced by the oxidation. Potassium periodate is essentially neutral to methyl red and is readily available in very pure form. It is sufficiently soluble in water to allow preparation of the 0.02 *M* solution.

The determination of periodate in the presence of iodate is based on the fact that periodates liberate iodine from iodides in weakly alkaline solutions but iodates do not. The liberated iodine may be titrated in alkaline solutions with arsenite but not with thiosulfate. The reaction on iodides requires a few minutes for completion. This delayed reaction helps to avoid any tendency to overtitrate at the end point.

Free iodine in the presence of sodium bicarbonate might be expected to react with acetaldehyde and yield iodoform. Tests on dilute solutions obtained from propylene glycol failed to show any such interfering side reactions, however.

Potassium periodate, usually written  $\text{KIO}_4$  for the dry salt, is neutral to methyl red but acid toward other indicators in aqueous solutions. Titration with sodium hydroxide to thymolphthalein end point (about pH 10) shows it to be a monobasic acid corresponding to the following reaction:



Potassium iodate is also neutral to methyl red but is not an acid. Under certain conditions this procedure may be used to determine periodate in the presence of iodate. However, the end point to thymolphthalein was not sufficiently sharp in dilute solutions to permit accurate results.

Other methods do not differentiate iodate from periodate. They measure the change in total oxidizing power but include iodate as well as periodate in the determination.

*Determination of Aldehydes.*—For the purposes of this study, volumetric methods were considered more desirable than colorimetric or gravimetric procedures for aldehydes. Available volumetric methods include Ripper's (19) bisulfite procedure as modified by Clausen (20) for the determination of acetaldehyde. In this procedure the aldehyde is converted to its bisulfite complex in the presence of excess sodium bisulfite. The aldehyde complex is a sulfonate that is stable in acid solutions. Excess sulfite may, therefore, be destroyed with iodine without affecting the aldehyde compound. In alkaline solutions the aldehyde sulfonate dissociates into free

aldehyde and sulfite. Under proper conditions the liberated sulfite may be titrated with iodine and is a measure of the aldehyde.

If sufficient excess bisulfite is used, periodates do not interfere in the procedure. Both iodates and periodates are reduced to iodide.

For acetaldehyde, Clausen (20) recommends sodium bicarbonate to produce the proper alkalinity for the final iodometric titration. However, in the presence of sodium bicarbonate the titration of formaldehyde sulfonate was too slow to be of practical use. It required about 45 minutes for a titration with 20 ml. of 0.02 *N* iodine. The titration of acetaldehyde on the other hand could be made quite rapidly.

The dissociation constants of the aldehyde complexes appear to be related to the rate at which they may be titrated. These constants have been reported in the literature (21) as  $1.2 \times 10^{-7}$  for formaldehyde sodium sulfite and  $2.5 \times 10^{-8}$  for acetaldehyde sodium sulfite, both at 25°C. The dissociation may be increased by raising the temperature and by increasing the alkalinity.

It was found that the borax-sodium carbonate mixture increased the alkalinity so that both the formaldehyde and acetaldehyde sulfonates could be titrated rapidly. Clausen (20) warned against the use of strong alkalis (such as sodium carbonate) because they caused the formation of iodoform as a side reaction with acetaldehyde. However, tests showed no significant interference from this source when the borax-sodium carbonate mixture was used. Although a starch-iodine end point with acetaldehyde is not entirely stable with either bicarbonate or borax-sodium carbonate buffer, it is stable long enough (about 1 minute) to be perceived sufficiently well for precise determinations. The blue color with formaldehyde is quite stable.

The aeration method for the isolation of acetaldehyde is based on the work of Nicolet and Shinn (15), (16), who used a similar procedure in the determinations of methyl pentoses and threonine. They found that acetaldehyde was readily expelled from aqueous solutions saturated with sodium bicarbonate. They used an amino acid (alanine) to prevent carry-over of traces of formaldehyde. Aminoacetic acid was more readily available than alanine and was found suitable for this purpose. It reduced the blank for carry-over of formaldehyde from about 0.7 to 0.0 ml. of 0.02 *N* iodine.

Another volumetric method for the determination of total aldehydes has been described for use in connection with the periodate oxidations (22), (18). This method depends on the reaction of aldehydes with excess sodium sulfite, whereby the free sodium hydroxide liberated can be titrated with standard acid and thymolphthalein. Excess periodate is reduced to iodate and does not interfere in the titration. However, experiments showed that the thymolphthalein end point was not sharp enough for precise results on dilute solutions.



## APPLICATIONS OF METHODS

The methods described have been applied to known amounts of glycerol, ethylene glycol, and 1,2-propylene glycol singly and in various combinations. The results of such tests are shown in Table 2. The reported recoveries are based on calculations from the individual determinations as indicated in the table. Table 1 shows the relationships that form the basis for calculations on mixtures.

TABLE 1.—Comparative volumes of 0.02 *N* reagents required by individual alcohols

	0.02 <i>N</i> NaOH (FORMIC ACID)	0.02 <i>N</i> ARSENITE (REDUCED KIO <sub>4</sub> )	0.02 <i>N</i> IODINE (TOTAL ALDEHYDES)	0.02 <i>N</i> IODINE (ACETALDEHYDE)
Glycerol	1	4	4	0
Ethylene glycol	0	1	2	0
Propylene glycol	0	1	2	1

With pure glycerol the formic acid titer in ml. of 0.02 *N* sodium hydroxide will be  $\frac{1}{4}$ th of the reduced potassium periodate in terms of 0.02 *N* arsenite and also  $\frac{1}{4}$ th of the 0.02 *N* iodine titer obtained in the determination of total aldehydes.

In a mixture containing glycerol and ethylene glycol, four times the formic acid titer is subtracted from the volume of 0.02 *N* iodine or from 0.02 *N* arsenite to correct for the effect of glycerol. The balance of iodine or arsenite may then be calculated to ethylene glycol.

TABLE 2.—Application of methods to known mixtures of glycerol, ethylene glycol, and propylene glycol

CONTAINED	RECOVERY BASED ON DETERMINATION OF—				
	AMOUNT OF KIO <sub>4</sub> REDUCED	FORMIC ACID	FORMALDEHYDE	ACETALDEHYDE	
	mg.	per cent	per cent	per cent	per cent
1. Glycerol	10.7	99.8	98.3	—	—
2. Glycerol	42.6	99.8	99.4	99.1	—
3. Ethylene glycol	10.7	99.8	—	—	—
4. Ethylene glycol	42.9	100.0	—	99.4	—
5. Propylene glycol	14.5	99.9	—	—	97.9
6. Propylene glycol	57.9	100.6	—	98.9	99.5
7. { Glycerol	17.1	—	98.8	—	—
{ Ethylene glycol	21.5	98.2	—	99.1	—
8. { Glycerol	17.1	—	98.8	—	—
{ Propylene glycol	19.3	98.5	—	97.0	98.0
9. { Glycerol	17.1	—	98.3	—	—
{ Ethylene glycol	21.5	97.9	—	98.2	—
{ Propylene glycol	19.3	—	—	—	99.5
10. { Ethylene glycol	21.5	97.9	—	97.9	—
{ Propylene glycol	19.3	—	—	—	99.5

The determination of acetaldehyde is used as the basis for the estimation of propylene glycol. In the presence of glycerol, ethylene glycol, and propylene glycol, the glycerol is calculated from the formic acid titration and propylene glycol from the acetaldehyde titration. Ethylene glycol may then be calculated in two ways:

(1) Subtract 4 times the formic acid titer and the acetaldehyde titer from the arsenite titer. The difference multiplied by 0.62 gives mg. of ethylene glycol, or (2) subtract 4 times the formic acid titer and 2 times the acetaldehyde titer from the total aldehyde titer. The difference multiplied by 0.31 gives mg. of ethylene glycol.

The proposed procedures have been found useful in the examination of the saponification products of synthetic fats and waxes, such as glycerol and glycol esters of fatty acids, and for the determination of these polyhydroxy alcohols in cosmetic mixtures.

Preliminary investigations have been made on sorbitol sirup and on mono- and diethanolamines that react with periodate. Tests have failed to confirm the literature statement (12) that diethanolamine yields 4 mols of formic acid.

Pure triethanolamine does not react with periodate. However, the commercial material used in cosmetics contains varying quantities of the reactive mono- and di- compounds. Diethyleneglycol, diethyleneglycol monoethylether, ethyl alcohol, sodium lactate, and sodium citrate are among the compounds that showed no reaction to periodate.

#### SUMMARY

A review of the literature on periodate oxidations shows numerous procedures of value in the analysis of cosmetics. Certain features of various published methods have been selected and tested for their applicability to the determination of small quantities of glycerol, ethylene glycol, and propylene glycol. The results of such tests on control samples of these alcohols have been included.

The proposed methods have also been of value in the study of the periodate oxidation on other organic compounds used in the formulation of cosmetics.

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## NOTE ON MICROSCOPIC IDENTIFICATION OF FERROUS SULFATE IN MIXTURES

By GEORGE L. KEENAN and WILLIAM V. EISENBERG\* (U. S. Food and Drug Administration, Washington, D. C.)

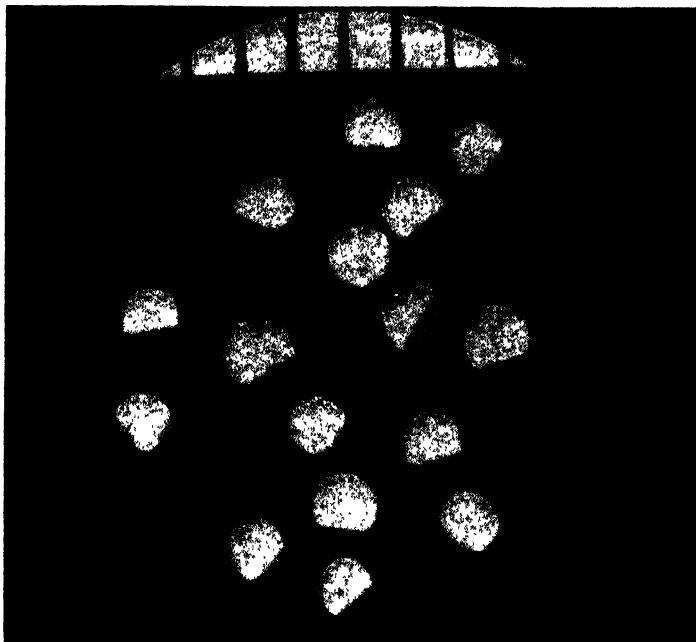
It is not uncommon to find in mixtures with other ingredients, and especially in drug and veterinary preparations of a granular character, a partially dried and oxidized ferrous sulfate. Frequently many of these crystals have the appearance of small, brown, eight-sided forms closely resembling octahedra, but more strictly in the monoclinic system they would be called bipyramids. The accompanying photomicrograph illustrates the general appearance of the material when found in such mixtures.

Normally, ferrous sulfate crystallizes with 7 H<sub>2</sub>O in the monoclinic system with the following indices of refraction:  $n_\alpha = 1.471$ ,  $n_\beta = 1.478$ ,  $n_\gamma = 1.486$ . A complete description of the salt is given in Groth's "Chemische Krystallographie," Vol. 2, pp. 431-433 (1908). In the mixtures referred to above, however, the salt frequently becomes partially dried and oxidized with resulting changes in refractive indices. This partially dried material may often be characterized by the indices 1.525 and 1.539, being the minimum and maximum refractive indices on crystal fragments showing the maximum amount of double refraction with crossed nicols (parallel polarized light). These data are quite useful in themselves in suggesting

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\* Contribution from Microanalytical Division.

the presence of ferrous sulfate. For confirmation, the partially dried material can be recrystallized from a drop of water on a microscopic slide and the indices for the heptahydrate readily determined. The frequency with which the altered form of ferrous sulfate occurs in such mixtures makes this technic for identifying the substance useful in analytical work.



Typical Forms, Ferrous Sulfate, Partially Dried  
( $\times 7$ )

#### MICROSCOPIC METHOD FOR DETECTION OF INSECT EXCRETA IN FLOUR AND MEAL

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There are two general methods by which insect excreta in flour may be counted. One utilizes sedimentation of the excreta in a heavier than water liquid, which floats the flour, and the other consists in so treating the flour that the excreta are made visible in the matrix.

Several difficulties may be encountered in flotation procedures because different particles of flour vary in their specific gravity, depending upon the part of the grain from which they were taken. For example, the bran is heavier than the starch, and even similar parts vary in weight according

to the presence or absence of entrapped air or other factors. Insect excreta pellets similarly show a wide density range depending upon the type of food eaten, physiological condition of the insect, and the species or stage of the insect. A liquid with specific gravity 1.52 will support most flour particles, but in it many insect excreta pellets may rise into the flour layer. At sp. gr. 1.40, the insect pellets settle out quite well, but much floury material settles with the excreta. Because of these difficulties it is impractical to depend solely on specific gravity for separation.

Howard,<sup>1</sup> in 1939, stated "... the insect excreta pellets behave differently when moistened with an oil than do wheat flour granules. The latter tend to become clear and transparent when immersed in an oil medium, while the excreta pellets retain more or less their white, opaque appearance." Howard's method was to demonstrate the presence of excreta, but the mineral oil and/or olive oil did not clear the flour sufficiently to make all the pellets visible and the handling of the sample left pellet residues in the equipment. Moreover, it involved considerable manual manipulation, and therefore was unsuited to routine testing.

Blumberg<sup>2</sup> concluded that the refractive index is important in rendering flour particles transparent, and he found that with higher refractive indices the flour is less apparent than it is with lower indices. He then used clove oil, but this method is not applicable to routine quantitative analyses. The use of a compound microscope greatly lengthened the time needed per sample, and in using transmitted light certain details of structure were rendered less distinctly visible. Furthermore, the desired stereoscopic picture is difficult to obtain with the compound microscope.

Factors other than refractive index must be considered in making the flour transparent and the pellets visible by contrast. The oil must readily penetrate the flour and drive out most of the air, and it must not be too volatile or the prepared mounts will be unstable while an examination is being made. In actual practice clove oil has been found to satisfy the requirements of such an examination although with any of the oils certain phosphates and salts found in self-rising flour remain opaque in oil and may bear a superficial resemblance to fragments of excreta pellets while some fragments of cereal fail to clear completely and so resemble excreta. Mineral material will appear crystalline unless its crystals have been destroyed by grinding, but in this case the appearance will be dissimilar to insect excreta in that the excreta will be smooth and rounded while the minerals will be angular and have flat or irregular cleavage sides. Internally the excreta show a heterogeneous laminated appearance while the salts are homogeneous. The following method, which eliminates certain of the difficulties encountered in analytical procedures for the detection

<sup>1</sup> U. S. Food and Drug Administration, Mimeograph, Jan. 7, 1939.

<sup>2</sup> Columbia University College of Pharmacy thesis, June, 1939.

of insect excreta in flour, has been found to be quite satisfactory for routine analysis:

#### METHOD

1. Weigh 0.20 gram of flour on a tared, flat, glass disk 7-7.5 cm. in diameter (two disks may be balanced and used as pairs). Add clove oil and spread into a thin uniform layer, using sufficient oil to clear the flour and present a smooth surface of oil, but not so much that the mixture will flow off the disk. Place a wire grid over the disk and examine with a Greenough-type binocular microscope at 25-30 magnification.

2. *Optional procedure.*—In a multiple-sample schedule, tare 2-8 small numbered vials on each balance pan and weigh by shifting the weights from one side to the other. (If desired, a larger portion may be weighed into a beaker and some of the flour floated off in a  $\text{CHCl}_3$ -ether or  $\text{CHCl}_3$ -toluol mixture of sp. gr. 1.40 before being transferred to the filter paper.) Rinse the contents of each vial with  $\text{CHCl}_3$  or  $\text{CCl}_4$  onto a smooth-surfaced ruled filter paper in a Hirsch funnel. Transfer the paper to a Petri dish, flood with clove oil, and examine with a dark background and intense reflected light so that the pellets stand out as whitish or opaque masses.

The mineral oil method described by Howard (loc. cit.) was used by Cotton et al.<sup>8</sup> in one investigation, from which it was concluded that “. . . ordinary tests to determine the condition of rebolted flour . . . will not necessarily reveal the fact that it has been heavily infested with insects over a considerable period of time, and therefore flotation tests and the flour-oil test must be employed to determine the presence of insect contamination.” More recently the multiple-sample procedure described above was used on 73 samples of rye flour and meal and on 81 samples of whole wheat flour and meal. In spite of the small sample it was found to be a satisfactory direct method for the quantitative detection of insect contamination.

#### FALSE POSITIVE PHOSPHATASE TESTS

By EMANUEL KAPLAN (Division of Chemistry, Bureau of Laboratories,  
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For more than five years the Bureau of Laboratories in the Baltimore City Health Department has used the Gilcreas-Davis modification (1) of the Kay-Graham phosphatase test (2) as an aid in the control of the pasteurization of milk and cream. This procedure is a tentative method of the Association of Official Agricultural Chemists (3).

In working in close collaboration with the Bureau of Milk Control this laboratory experienced difficulty with the phosphatase test for the first time in November, 1939, when a false positive reaction was obtained. This false positive test was found to have been caused by a thermostable sub-

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<sup>8</sup> *American Miller*, 69, 10, 34-39 (1941).

stance capable of hydrolyzing the buffered substrate used in the test. Since that time, as a safeguard against any possible misinterpretation of laboratory results on the part of the Bureau of Milk Control, all samples yielding phosphatase readings indicative of improper pasteurization were routinely repasteurized in the laboratory. In this way heat-resistant interfering substances were recognized. During the period between November, 1939 and September, 1942, 21 samples of milk and cream showing a false positive reaction were detected in the examination of more than 4500 commercially pasteurized samples. No regulatory action was taken in any of these 21 instances. Only about one per cent of the total samples examined showed a positive test, yet 50 per cent of all positive tests noted in this period were judged to be false positives.

In December, 1941, a probable cause of the false positive phosphatase tests was finally established with the isolation of a hitherto unidentified thermophilic bacillus of the genus *Lactobacillus* from a suspected sample of commercial pasteurized milk. The morphological, biochemical, and cultural characteristics of this organism, as well as its ability to produce a thermostable phosphatase enzyme, have been reported by Buck (4). The production of false positive phosphatase tests is illustrated in the following experiment based partly on data already described (4).

One quart of low count certified raw milk was transferred to a covered sterile beaker equipped with motor-driven agitation and partially immersed in a water bath. The milk, inoculated with a culture of *Lactobacillus enzymothermophilus* (4), was maintained at a temperature of 142°–143°F. for 5½ hours. During this period samples were periodically removed for examinations. Phosphatase tests were made by both the Gilrease (1) and the Scharer (5) methods. The pH of the milk was determined by means of the glass electrode. Boiling or clot tests (6) were done by heating 3–5 ml. of milk in a boiling water bath for 5 minutes. Total bacterial counts were obtained by the direct microscopic method (7).

The results of these examinations are summarized in Table 1.

It is apparent from the above experiment that the growth of this thermophilic organism resulted in the production of a thermostable substance showing phosphatase activity as measured by the two phosphatase tests employed. Portions of the milk in the above experiment were further heated at the conclusion of the 5½ hour holding period. A temperature of 180°F. for 30 minutes had little effect on the phosphatase activity. The activity was destroyed after several minutes heating in a boiling water bath.

#### CONTROL TESTS

The possibility of false positive phosphatase tests as a result of the presence in milk of certain types of bacteria capable of hydrolyzing the substrate used in the test has been suggested by the experiments of various investigators (4, 6, 8, 9, 10). Leahy (8) points out that phosphatase tests made upon samples of milk having microscopic counts higher than

TABLE 1.—*Production of false positive phosphatase tests by Lactobacillus enzymothermophilus* (4) at 142°–143° F.

PHOSPHATASE TESTS							
HOLDING TIME  (minutes)	GILCREAS UNITS		SCHARER UNITS		pH	CLOT TEST	TOTAL BACTERIAL COUNT (DIRECT MICROSCOPIC)
	TEST	UNINCUBATED CONTROL	NO pH ADJUSTMENT	pH ADJUSTMENT*			
Uninoculated milk	0.70	0.01	—	—	6.55	Neg.	—30,000
Inoculated milk	0.70	0.01	—	—	—	—	60,000
10	0.40	0.01	—	—	—	—	—30,000
20	0.18	—	Over 10	—	—	—	35,000
30	0.06	0.01	5	—	6.55	Neg.	60,000
90	0.015	—	1.5	—	6.50	Neg.	150,000
150	0.05	—	2.5	3.0	6.50	Neg.	7,800,000
210	0.11	0.01	5.0	6.0	6.20	Neg.	39,000,000
270	0.20	—	6.0	7.5	6.10	Clot	99,000,000
330	0.25	0.01	2.0	7.5	5.90	Clot	150,000,000

\* Milk adjusted to pH by means of Na<sub>2</sub>CO<sub>3</sub> solution before testing

8,000,000 per ml. or standard plate counts higher than 2,000,000 per ml. should be interpreted with caution. On the basis of experimental findings, Neave (6, 9) concluded that bacterial interference in the test was unlikely unless the organisms were present in sufficient numbers to cause the milk to clot on boiling or to produce a bacterial taint. Neave emphasized that such milk should be excluded from testing. Burgwald (10) also concluded that there was little likelihood of bacterial interference with the test, and notes that if bacteria were present in sufficient numbers to cause errors, the use of control tests would indicate the condition. However, he does not state the nature of the control tests to be applied in such instances.

The usual control tests in phosphatase methods for the determination of pasteurization (7, 11) are designed to check deterioration of reagents and to note the presence of interfering substances in the sample. These control procedures are in the nature of unincubated controls and hence do not indicate the presence of thermostable substances of bacterial origin showing a phosphatase activity. In order to recognize false positive reactions the following procedure is routinely followed in this laboratory whenever a sample shows a phosphatase reading indicative of possible improper pasteurization.

The usual reagent control and the control on an unincubated portion of the sample are carried out in order to eliminate preformed phenolic substances and other interferences of this type. The sample is repasteurized in the laboratory. A 5 ml. portion of the suspected material is transferred to a test tube and then maintained in a water bath for 30 minutes at a temperature in the sample of ca. 145° F. The milk or cream is frequently stirred by means of an accurate thermometer, the bulb of which is kept constantly immersed in the sample. A phosphatase test as well



as an unincubated control is then made on the repasteurized portion. If there is no significant lowering of the phosphatase reading as a result of the repasteurization procedure, the original test is judged "false positive" and is presumed not to be associated with faulty pasteurization technic. Wherever possible, total bacterial plate counts and direct microscopic counts are also made.

Table 2 shows the results obtained with the laboratory repasteurization control procedure in the examination of ten samples of milk and eleven samples of cream in which the phosphatase tests were judged to be falsely positive.

TABLE 2.—*Effect of laboratory repasteurization control procedure on phosphatase readings—November, 1939, to September, 1942*

PHOSPHATASE READINGS (GILCREAS UNITS)							
MILK				CREAM			
BEFORE REPAST.		AFTER REPAST.		BEFORE REPAST.		AFTER REPAST.	
TEST	UNINCUBATED CONTROL	TEST	UNINCUBATED CONTROL	TEST	UNINCUBATED CONTROL	TEST	UNINCUBATED CONTROL
0.12	0.01	0.12	0.02	0.05	0.01	0.07	0.01
0.12	0.02	0.14	0.01	0.06	0.01	0.05	—
0.05	0.02	0.06	0.02	0.05	0.01	0.04	0.01
0.06	0.02	0.05	—	0.08	0.01	0.12	0.01
0.07	0.02	0.06	—	0.05	0.01	0.05	0.01
0.13	0.01	0.13	—	0.05	0.01	0.06	0.01
0.15	0.01	0.15	0.01	0.05	0.01	0.05	0.01
0.09	0.01	0.10	0.01	0.05	0.01	0.05	—
0.07	0.01	0.06	—	0.05	0.01	0.04	0.01
0.08	0.02	0.07	0.02	0.05	0.02	0.05	0.02
—	—	—	—	0.05	0.03	0.05	0.03

Because of the widespread use of the phosphatase test in regulatory work (12), samples showing an original positive phosphatase test should be retested as directed above to eliminate the misinterpretation of a positive test that could have been caused by thermostable substances showing phosphatase activity.

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## STUDY OF FACTORS INFLUENCING A.O.A.C. CHICK METHOD OF VITAMIN D ASSAY

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Since its official adoption,<sup>1</sup> a number of changes that have increased the accuracy and practical efficiency of the method have been made in the A.O.A.C. vitamin D chick assay. Lack of uniformity, which can still be found in the results obtained with this bioassay, may arise from three main sources: variation among chicks, inadequacy of the basal diet, and treatment of the bones. The following critical studies have been directed towards the possibility of improvement of the present procedure:

- I. Influence of the number of chicks at each level of vitamin D on the accuracy of the method.
- II. Influence of different breeds on the accuracy of the method.
- III. Influence of different strains of yeast in the basal ration.

### I. INFLUENCE OF NUMBER OF CHICKS AT EACH LEVEL

In early experiments carried out by Lachat *et al.*,<sup>2</sup> leading to the adoption of the bone ash method of vitamin D assay, groups of from 6 to 22 chicks per level of vitamin D were used to determine the average response.

In the subsequent revisions of the method,<sup>3,4</sup> groups of 10 or more chicks were specified for each level. Recently, the tentative method<sup>5</sup> recommended the use of 20 chicks per level at the beginning of the feeding period in order to be certain of having bone ash values for at least 15 chicks at the conclusion.

From the data supplied by 13 laboratories, Oser<sup>6</sup> calculated the standard error for the mean of the individual ash values falling in the region of maximum sensitivity in the bone ash response curve. When a degree of

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<sup>1</sup> *This Journal*, 17, 68-9 (1934).

<sup>2</sup> *Ibid.*, 15, 660-76 (1932).

<sup>3</sup> *Ibid.*, 17, 69 (1934).

<sup>4</sup> *Ibid.*, 18, 72-3 (1935).

<sup>5</sup> *Ibid.*, 22, 81-2 (1939).

<sup>6</sup> *Ibid.*, 446-49.

certainty of 95 per cent was used as a criterion of significance, a minimum significant difference of 2.73 per cent was found between mean bone ash values calculated for groups of 10 chicks. Since the standard error of a mean is inversely proportional to the square root of the number of observations, the increase in accuracy resulting from successive equal increments in the number of observations becomes progressively smaller. Thus, for example, to halve the minimum significant difference it is necessary to quadruple the number of observations. This factor, as well as the additional cost involved, must be considered when the group size is increased.

The extent of variation among individual bone ash values may be expected to differ for various laboratories, and it is advisable, as pointed out by Oser, that each investigator determine the precision of his assays and thereby establish a basis for choosing the optimum group size for use under his conditions. Accordingly, a statistical examination was made of 440 bone ash values obtained in this laboratory. All chicks received the same vitamin D supplement\* at varying levels, and the bone ash values were obtained by the technic of the tentative A.O.A.C. method.<sup>†</sup> In this and in all succeeding experiments, the chicks were kept in an air-conditioned room at a thermostatically controlled temperature of 80°F.

Standard deviations for bone ash values at five levels of vitamin D are given in Table 1. The values obtained at the 10, 15, and 22.5 unit levels were considered to be representative of the region of greatest sensitivity in the dosage response curve and were averaged. A statistical test indicated that these values did not differ significantly.

TABLE 1.—Standard deviations of per cent bone ash values of chicks on different levels of vitamin D

UNITS OF VITAMIN D PER 100 GRAMS OF FEED	MEAN PER CENT BONE ASH	NUMBER OF CHICKS	ST. DEV.* OF PER CENT BONE ASH	AV. ST. DEV. FOR REGION OF MOST SENSITIVE RESPONSE
4.44	33.4	80	2.76	
10	36.6	100	3.41	
15	38.8	100	3.98	3.63
22.5	41.5	100	3.47	
33.75	45.3	60	2.91	

\* Standard Deviation =  $\sqrt{\frac{NS(x^2) - (\Sigma x)^2}{N(N-1)}}$ ; where  $N$  = number of chicks.

† The average standard deviation is the square root of the average of the three variances at 10, 15, and 22.5 unit levels of vitamin D.

The method of determining the significance of a difference between

\* Canadian Standard Reference Cod Liver Oil containing 115 U.S.P. units of vitamin D per gram.

† *Methods of Analysis*, A.O.A.C., 1940, 371.

means involves comparing the difference with the standard error. The standard error of the difference is:

$$\text{s.e.}_{\text{diff.}} = \sqrt{\frac{(\text{s.d.})_1^2}{N_1} + \frac{(\text{s.d.})_2^2}{N_2}},$$

where s.d. = standard deviation. A difference as great or greater than twice its standard error would arise from errors of random sampling alone only once in 22 sampling trials, and such a criterion of significance seems adequate for use with a biological assay. If a criterion that the minimum significant difference should be twice its standard error is accepted, then  $2 = (\text{difference}/\text{s.e.}_{\text{diff.}})$ ; or, if it is assumed that the bone ash values of both the standard and the assay oil have the same error, difference  $= 2\sqrt{2/N}(\text{s.d.})$ .

Table 2 gives the minimum significant difference for various group sizes, calculated for the region of most sensitive response in the dosage response curve by substituting the value s.d. = 3.63 and the appropriate values of "N" in the expression  $2\sqrt{2/N}(\text{s.d.})$ . Such values represent the minimum difference in per cent bone ash that may be interpreted as indi-

TABLE 2.—Minimum significant differences between per cent bone ash values for various group sizes

NUMBER OF CHICKS PER GROUP	DATA OF MOTZOK ET AL.	DATA OF OSER
20	2.30	1.93
30	1.88	1.58
40	1.62	1.37
50	1.45	1.22
60	1.33	—
80	1.15	—
100	1.03	0.86

cating a difference in potency between a standard and an assay oil. The data of Oser, which he calculated in a similar way from the standard error of the mean for bone ash values falling on the steepest part of the curve, are included for comparison.

These data (Table 2) show that minimum differences obtained in this laboratory for the region of maximum response are considerably higher than those calculated by Oser. For example, to obtain an accuracy equivalent to that calculated by Oser for groups of 20 chicks a group size of approximately 30 is required. This emphasizes the suggestion that different investigators should establish their own standards of precision. It is apparent that a group size of 15 chicks, as recommended by the A.O.A.C., does not give a very high degree of precision under the conditions in this laboratory, and accordingly the practice of using approximately 35 birds

per level has been adopted. Some of the work reported in the following sections of the present paper was conducted before this change was made, and consequently in these experiments smaller group sizes were used.

## II. INFLUENCE OF DIFFERENT BREEDS

The A.O.A.C. recommends the use of White Leghorn chicks in the bioassay of vitamin D supplements. Griem *et al.*<sup>8</sup> have shown that this breed gives uniform assay results even though the birds are obtained from different commercial hatcheries. However, one of the greatest sources of error is the difference in the response of individual chicks to a given level of vitamin D. This has necessitated the use of a large group at each level of the graded doses in order to establish the bone ash response with a reasonable degree of certainty.

The degree of variation among individual chicks in their per cent bone ash at a given level of vitamin D may be an inherited characteristic in any one breed. Other factors being equal, a pure breed or hybrid giving a more nearly uniform response than do White Leghorn chicks would be more suitable for use in the A.O.A.C. method. The following preliminary report presents the data on six pure breeds and three hybrids in regard to their suitability for the chick method of vitamin D assay. The bone ash values obtained during two consecutive seasons and coefficients of variation are presented in Tables 3 and 4. In the earlier work the White Leghorns were compared with Barred Plymouth Rocks, New Hampshires, and Hybrid A obtained by back crossing New Hampshire males with the females of reciprocal crossing of New Hampshire and New Ontario breeds. In the second series, studies were conducted on White Leghorns, Barred Plymouth Rocks, New Hampshires, Light Sussex, Black Jersey Giants, White Wyandottes, Hybrid B (Brown Leghorns  $\times$  Barred Plymouth Rock), and Hybrid C (New Hampshire  $\times$  Barred Plymouth Rock). Except for the use of different breeds of chicks, the technic of the A.O.A.C. method was employed.

A comparison of the total spread in per cent bone ash between 0 and 22.5 unit levels of vitamin D (Table 3) indicates that the New Hampshire breed compared favorably with the White Leghorns, whereas the Barred Plymouth Rock breed gave a somewhat smaller spread. This relationship for the three breeds is substantiated by the data presented in Table 4, since the Barred Plymouth Rocks again showed the least sensitive response. Of the other breeds the Light Sussex gave a spread of 6.1 per cent, which was only slightly less than that given by White Leghorns, while the Black Jersey Giants and White Wyandottes gave considerably lower values. However, the data indicate that the present experiment did not cover the region of most sensitive response for the last three breeds, and

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<sup>8</sup> *This Journal*, 18, 471-75 (1935).

it will be necessary to extend the range of graded vitamin D dosage in order to make a satisfactory appraisal of the suitability of these breeds.

In the tests of both seasons the coefficients of variation for White Leghorn chicks were found to be consistently higher over the steep portion

TABLE 3.—*Influence of breed of chicks on bone ash response to various levels of vitamin D (1941 season)*

BREED	NUMBER OF CHICKS PER GROUP	UNITS OF VITAMIN D PER 100 GRAMS OF RATION	MEAN PER CENT BONE ASH	SPREAD IN PER CENT BONE ASH	COEFFICIENT OF VARIATION
White Leghorn	18	0	30.5	13.6	10.82
	18	4.44	32.4		11.20
	18	10	39.2		13.11
	17	12.25	42.9		7.29
	20	15	44.4		7.36
	18	18.37	44.8		7.32
	17	22.5	44.1		6.96
Barred Plymouth Rock	17	0	29.5	11.6	5.15
	18	4.44	30.6		9.28
	17	10	35.4		10.00
	19	12.25	35.2		8.32
	18	15	37.6		8.75
	20	18.37	41.0		7.70
	15	22.5	41.1		5.49
New Hampshire	15	0	30.7	13.3	5.05
	17	4.44	34.2		8.56
	15	10	37.5		6.88
	16	12.25	38.3		10.02
	17	15	40.6		8.62
	16	18.37	42.9		7.36
	17	22.5	44.0		5.75
Hybrid A	15	10	37.5		4.16
	15	15	40.5		9.02
	15	22.5	44.1		3.52

of the dosage response curve than they were for the other breeds. From the data in Table 3 it can also be seen that Hybrid A gave the low coefficient of variation of 4.16 at a calcification of 37.5 per cent bone ash. Further evidence that a smaller variation can be expected among cross-bred chicks is presented in Table 4. These data show that when comparisons are made at approximately the same degree of calcification Hybrid B and Hybrid C give less individual variation than do the White Leghorn chicks, the coefficient of variation for the individuals of Hybrid C being the lowest of all the breeds studied.

It has long been observed by both plant and animal breeders that the  $F_1$  hybrids from inbred lines show a remarkable degree of uniformity in all physical characteristics as compared with normal cross-bred stock. This suggests that a decrease in variability in bone ash response may be obtained by the crossing of two breeds, each of which is relatively homozy-

TABLE 4.—*Influence of breed of chicks on bone ash response to various levels of vitamin D (1942 season)*

BREED	NUMBER OF CHICKS PER GROUP	UNITS OF VITAMIN D PER 100 GRAMS OF RATION	MEAN PER CENT BONE ASH	SPREAD IN PER CENT BONE ASH	COEFFICIENT OF VARIATION
White	39	10	36.0		11.5
Leghorn	38	15	38.7	6.7	8.95
	35	20	42.7		6.76
Barred	36	10	34.7		6.55
Plymouth	32	15	37.0	5.0	8.94
Rock	24	20	39.7		8.13
New	24	10	35.3		8.45
Hampshire	23	15	41.3	7.6	7.37
	22	20	42.9		5.58
Light	21	10	32.5		6.03
Sussex	22	15	34.2	6.1	9.62
	19	20	38.6		6.26
Black	17	10	33.9		8.17
Jersey	19	15	35.9	3.4	7.60
Giant	24	20	37.3		7.45
White	14	10	38.3		7.55
Wyandotte	17	15	40.7	3.7	6.41
	18	20	42.0		5.30
Hybrid B	40	15	35.2		8.49
Hybrid C	42	15	36.9		6.88

gous in different factors that influence the degree of response to graded doses of vitamin D. The data presented in this paper indicate that cross-breeding of two inbred lines may reduce individual variability to an appreciable extent. Further investigation with cross-breeds and the use of larger numbers of birds and different crosses may lead to the development of a hybrid possessing a low degree of variability and a favorable range of sensitivity to vitamin D intake. The use of such a hybrid would increase the accuracy and practical efficiency of this bioassay.

### III. INFLUENCE OF DIFFERENT STRAINS OF YEAST IN BASAL RATION

Several changes have been made in the official specifications for the yeast content of the diet.<sup>1,2,4</sup> The most recent recommendation<sup>5</sup> specified the use of 2 per cent of non-irradiated yeast containing a minimum of 7 per cent nitrogen. No qualification was made, however, regarding the strain or commercial source to be used.

In the basal ration, the yeast provides a number of nutritional factors including proteins and several vitamins of the B complex. Owing to the differences in strains and methods of production, commercial yeasts may vary considerably in their feeding value. Accordingly, a study was made of the influence on the assay of four strains of commercial non-irradiated yeasts\* produced by different cultural methods. The riboflavin content of these yeasts as determined by the Snell and Strong microbiological method<sup>9</sup> was as follows: Strain A, 39 p.p.m.; Strain B, 56 p.p.m.; Strain C, 38 p.p.m.; Strain D, 74 p.p.m. Approximately 400 White Leghorn chicks were used, and each yeast was supplemented with five levels of vitamin D, 4.44, 10, 15, 22.5, and 33.75 units per 100 grams of basal, respectively. Other details of the method were in accordance with the A.O.A.C. recommendations.

The bone ash responses for the four yeasts are presented graphically in Figure 1. From these data, it can be seen that Strains A and B produced more nearly uniform increases in calcification with increasing doses of vitamin D than did Strains C and D. At the lower levels of vitamin D there was considerable variation in all of the curves, but at 22.5 and 33.75 unit levels the bone ash values for the first two strains were from 1 to 3 per cent higher than for the other two.

The influence of the yeast on the growth of the chicks varied considerably (Figure 2). The chicks fed on rations containing yeast A and B made more nearly uniform and considerably higher gains than did those receiving yeast C and D. Computations based on the results of assays on the ingredients of the rations showed that the diet with yeast D contained approximately 330 micrograms of riboflavin per 100 grams. This value approximates the optimum riboflavin requirements of chicks as established by Norris<sup>10</sup> and Titus.<sup>11</sup> The diet with yeast A had a riboflavin content somewhat below the minimum requirements and yet it enabled the chicks to make better gains than did the ration with yeast D. From these observations, it does not seem probable that the differences in growth could be attributed to the riboflavin content of the yeasts. Apparently, some essential factor or group of factors other than riboflavin

\* Supplied through the courtesy of Anheuser-Busch Inc., St. Louis, Mo. and Meade Johnson, Belleville, Ontario.

<sup>1</sup> *Ind. Eng. Chem.*, 11, 346 (1939).

<sup>2</sup> *Poultry Tribune*, 20 (1942).

<sup>4</sup> "Food and Life," U.S.D.A. Yearbook 1939.



is concerned with the observed variations in growth. However, it should be pointed out that the riboflavin requirements established by Norris and Titus may not be applicable to the chicks used in this laboratory, and studies are in progress to determine whether the riboflavin content of the A.O.A.C. diet for vitamin D assay is adequate under these conditions.

It is also apparent from Figure 2 that optimum growth is reached at approximately 22.5 units of vitamin D per 100 grams of ration. The degree of calcification at this level of vitamin D is usually located at the upper

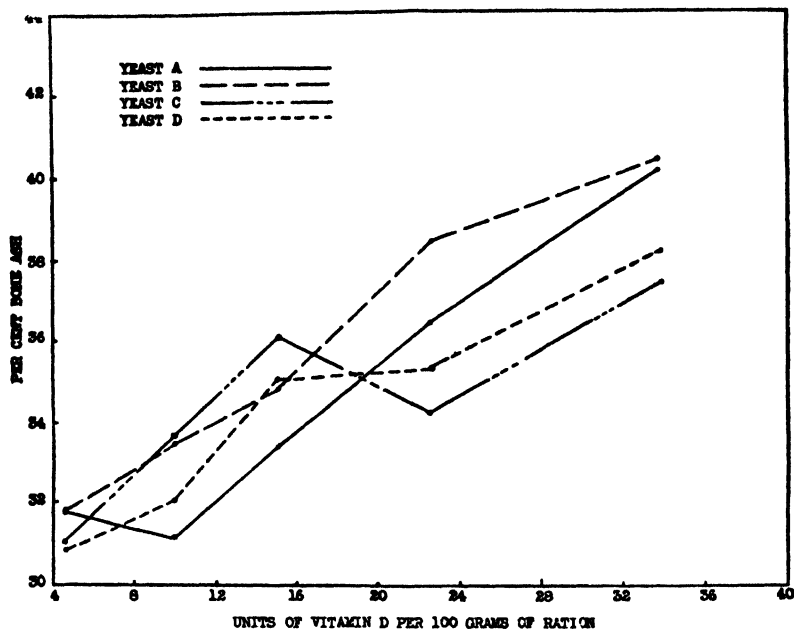


FIG. 1.—INFLUENCE OF STRAIN OF YEAST IN A.O.A.C. RATION ON BONE ASH RESPONSE AT FIVE LEVELS OF VITAMIN D.

part of the bone ash response curve, indicating that at levels of vitamin D generally used in the chick assay the basal ration is inadequate for optimum growth. From the data presented in Figures 1 and 2, it can be seen that the strains of yeast that gave good growth also favorably influenced the bone ash response.

If growth influences the degree of calcification to any great extent, considerable variation may be encountered in this method if there is distortion of the parallelism of the relationships of vitamin D and growth and vitamin D and calcification. Such a condition may be complicated further by differences in the efficiency of utilization of the feed as shown in Figure 3. When compared with the results on Yeast A, the other strains gave considerably lower and very irregular gains in weight per 100 grams of feed.

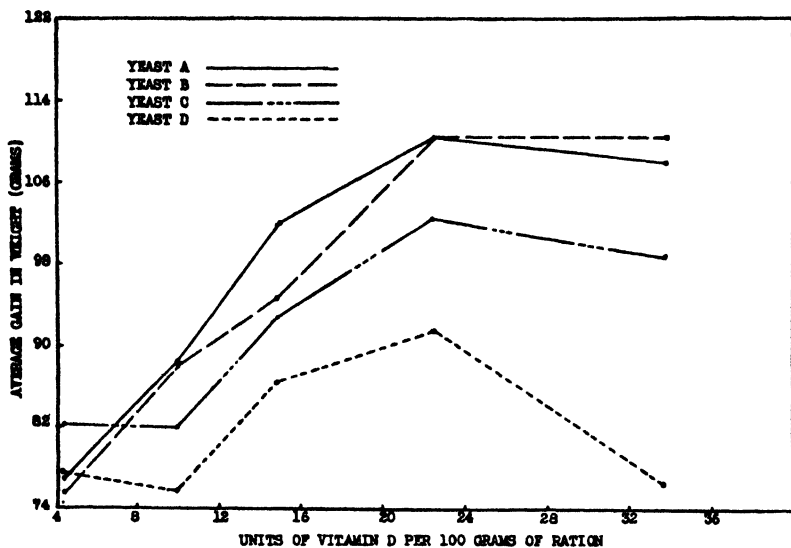


FIG. 2.—INFLUENCE OF STRAIN OF YEAST IN A.O.A.C. RATION ON GAIN IN WEIGHT OF CHICKS AT FIVE LEVELS OF VITAMIN D.

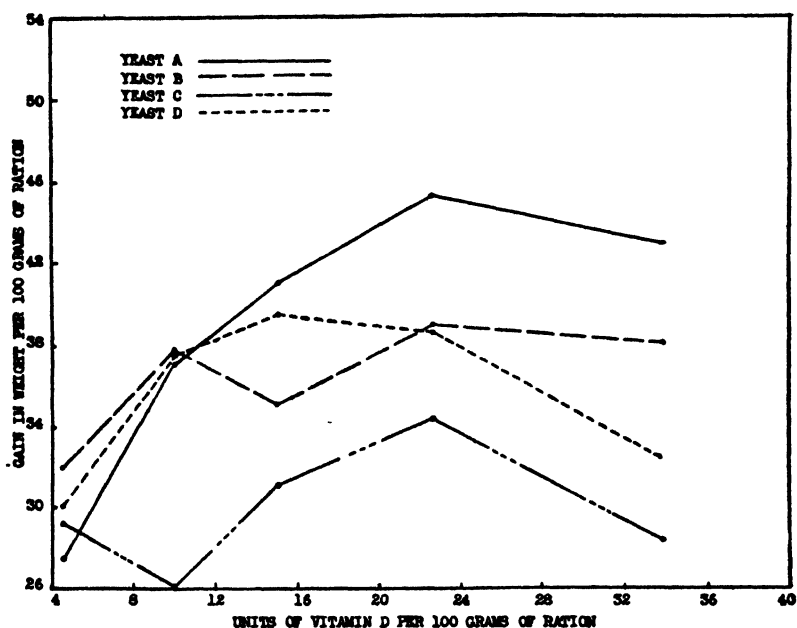


FIG. 3.—INFLUENCE OF STRAIN OF YEAST ON GAIN IN WEIGHT OF CHICKS PER 100 GRAMS OF RATION AT FIVE LEVELS OF VITAMIN D.

However, it can be seen from Figure 2 that the lower utilization of the ration containing Yeast B had little effect on the total gains made by the chicks as compared with Yeast A. The difference in the total intake of nutrients may account for the bone ash response being consistently higher with the ration containing Strain B than with the one containing Strain A.

In general, it is concluded that the choice of the strain of yeast may have a profound influence on the degree of calcification as affected by graded doses of vitamin D in the chick assay.

Further studies on the relationship of calcification, growth, and utilization of the ratio are indicated, and it is possible that a study of the influence of varying amounts of yeast in the basal diet may clarify some of the complexity of the problem.

#### SUMMARY

From the findings presented in this report, the following general observations are made:

1. In this laboratory, a group size of 15 chicks, as recommended by the A.O.A.C., does not give a very high degree of accuracy. The data given substantiate the suggestion that different investigators should establish their own standard of precision.
2. The breed of chick influences the type of response to graded doses of vitamin D. Of the breeds studied, White Leghorns showed the greatest variability. The uniform response found with cross-bred chicks suggests that a study of different crosses of inbred lines may produce a hybrid, the use of which, in the vitamin D assay, would increase the accuracy and practical efficiency of the method.
3. The choice of strain of yeast may have a marked influence on the degree of calcification, growth, and utilization of the ration as affected by graded doses of vitamin D. There is a suggestion that the basal diet may be deficient in a factor or group of factors necessary for growth. The apparent relationship between calcification and growth remains obscure, and further investigations are indicated.

#### ACKNOWLEDGMENT

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## DETERMINATION OF PARADICHLOROBENZENE IN SOIL

By R. D. CHISHOLM and L. KOBLITSKY (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture)\*

One method authorized by the United States Department of Agriculture (B.E.P.Q. 499) for the treatment of nursery stock to kill the Japanese beetle requires the use of paradichlorobenzene mixed with soil. The plant balls are plunged in this mixture, maintained within specified temperatures, and left undisturbed for several days. Upon completion of the treatment the paradichlorobenzene-soil mixture is discarded, and fresh mixtures are prepared for subsequent treatments. Not only does this procedure result in waste of the paradichlorobenzene, but it requires the procurement and preparation of large amounts of soil, especially at the large nurseries. This waste and the high labor cost have been unavoidable owing to the lack of a method for determining the residual paradichlorobenzene. With such a method it would be possible to add sufficient paradichlorobenzene to the used mixture to restore the original concentration and thus reduce the cost of the treatment.

Methods for the estimation of paradichlorobenzene based on the determination of chlorine would encounter serious difficulties if applied to such soil mixtures, chiefly because of the large proportion of soil in the mixtures and the presence of variable amounts of moisture and chlorine compounds in the original soil. The method described in this paper consists in the removal of the paradichlorobenzene from the soil mixture by steam distillation, its recovery in a solvent, and the determination of the difference between the refractive index of the solution and that of the solvent.

## METHOD

It was desirable to use a solvent of low volatility, and with a refractive index as different as possible from that of paradichlorobenzene. A refined kerosene with an index ( $n_D^{25}$ ) of 1.4330 was chosen.

The paradichlorobenzene-soil mixture was placed in a 1 liter boiling flask (round bottom, short ring neck), which was then closed with a two-hole rubber stopper equipped with two glass tubes and immersed to the neck in an oil bath. One tube, the lower end of which was covered with cheesecloth, extended from the bottom of the flask to a steam generator. The other tube extended from the lower surface of the stopper to a vertical 300 mm. Liebig condenser, and the lower end of the condenser tube projected into a 500 ml. separatory funnel containing 25 ml. of the refined kerosene.

The oil bath was heated to and maintained at a temperature close to

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\* The writers are indebted to S. R. Dutky for helpful suggestions and to F. M. Wadley for assistance in the statistical analysis of the data.

110°C. The paradichlorobenzene-soil mixture was steam-distilled until approximately 300 ml. of distillate had been recovered. Paradichlorobenzene that had crystallized in the condenser was collected in the receiver by shutting off the cooling water, thus allowing the inner tube to heat. The paradichlorobenzene was dissolved in the 25 ml. of kerosene by gentle shaking of the separatory funnel, the excess water was drawn off, and the solution was filtered.

The refractive index ( $n_D^{25}$ ) of the solution was measured by means of an Abbé type refractometer and compared with that of the refined kerosene.

### RESULTS

This procedure was applied to mixtures prepared by adding from 0.300 to 3.000 grams of paradichlorobenzene, in steps of 0.300 gram, to 500 ml. of soil. The refractive index differences found in these determinations involving six of the concentrations are shown in Table 1.

TABLE 1.—*Difference between the refractive index ( $n_D^{25}$ ) of kerosene, 1.4330, and that of kerosene solutions of paradichlorobenzene recovered from soil*

PARADICHLOROBENZENE, (GRAMS/25 ML. KEROSENE)		REFRACTIVE-INDEX DIFFERENCE ( $\times 10^4$ )						
0.300	10							
0.600	20	20	20					
1.200	40	38	38					
1.800	58	58	58	58	56	54	54	
2.400	74	74	72	72	72	72	72	72
3.000	92	92	92	92	90	90	90	90

The averages of these observations are plotted in Figure 1 in comparison with the results of another series of tests made without soil. In this other series concentrations ranging from 0.300 to 3.000 grams, in steps of 0.300 gram per 25 ml. total volume, were prepared, and the refractive indices were measured directly. It was found that these results could be expressed, with negligible error, by the following formula:

$$\text{Refractive-index difference} = 33.33 \left( \text{concentration in terms of g./25 ml. total volume} \right) \times 10^{-4}$$

On the basis of the observation that a solution made from 12 grams of paradichlorobenzene and 100 ml. of kerosene had a volume of 109.5 ml., and the assumption that smaller amounts of the solid would produce proportionate changes of volume, the coordinates of the points necessary to construct the graph shown in Figure 1 were calculated. The close clustering of the distillation results around this line indicates satisfactory recovery of the paradichlorobenzene.

Because the departure from linearity of the graph illustrating the

experiments from which soil was omitted is so slight, it was considered that the calibration graph summarizing the distillation experiments could be represented well enough by a straight line, and the line of best fit was determined by the method of least squares. The following relationship was found:

$$10^4 \text{ (refractive-index difference)} = 29.37 \text{ (g. paradichlorobenzene per 25 ml. kerosene)} + 2.80 \pm 1.4$$

Because standard nursery practice is to use concentrations based on

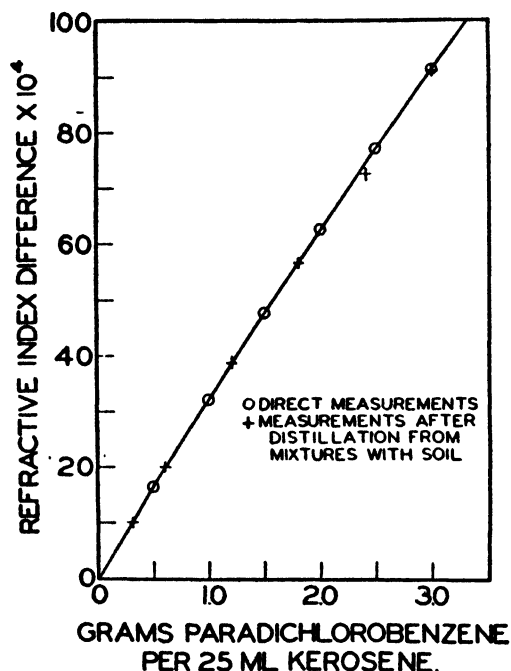


FIG. 1.—RELATION BETWEEN CONCENTRATION OF PARADICHLOROBENZENE IN KEROSENE SOLUTION AND DIFFERENCE BETWEEN ITS REFRACTIVE INDEX AND THAT OF KEROSENE.

“pounds per cubic yard,” and the application of the method involves estimation of concentration from measurements of refractive index, this regression formula is better transformed as follows:

$$\begin{aligned} \text{Pounds of paradichlorobenzene per cubic yard} = & 1148 \\ & (\text{refractive-index difference}) - 0.32 \pm 0.16 \end{aligned}$$

#### APPLICATION OF METHOD

Samples of six mixtures of paradichlorobenzene and sassafras sandy loam that had been used in the treatment of nursery plants were analyzed by the method described in this paper. By means of a thin steel tube a

sufficient number of 1 inch cores to equal approximately 3 liters were taken from leveled treating beds, mixed, and stored in air-tight containers. Cores were taken from each mixture, transferred to a graduated cylinder, and gently compacted, until a volume of 500 ml. was obtained. This 500 ml. sample was then transferred to the boiling flask, and the paradichlorobenzene was determined in duplicate.

The results of these determinations are presented in Table 2.

TABLE 2.—*Paradichlorobenzene found in soil mixtures that had been used in treatment of nursery plants*

SAMPLE	ORIGINAL CONCENTRATION (LBS./CUBIC YARD)	$n_D^{25}$ OF KEROSENE SOLUTION	DIFFERENCE FROM $n_D^{25}$ OF KEROSENE ( $\times 10^4$ )	FINAL CONCENTRATION, (LBS./CUBIC YARD)
1	10	1.4388	58	6.34
		1.4388	58	6.34
2	10	1.4380	50	5.42
		1.4384	54	5.88
3	8	1.4374	44	4.73
		1.4372	42	4.50
4	8	1.4374	44	4.73
		1.4372	42	4.50
5	6	1.4356	26	2.66
		1.4352	22	2.20
6	6	1.4352	22	2.20
		1.4352	22	2.20
Blank	0	1.4330	00	0.00

The extreme range between duplicates is 0.46 pound per cubic yard; the mean difference is 0.23 pound per cubic yard, which is equivalent to a refractive-index difference of 0.0002, the smallest step used in measuring the refractive indices of the solutions. These data indicate the precision that can be expected when the amount of paradichlorobenzene taken for a determination is subject to the variation in uniformity and the errors of sampling naturally inherent in these soil mixtures.

In Table 2 it is shown that the refractive index of the kerosene recovered after steam distillation of the soil with no paradichlorobenzene present (blank) was the same as the refractive index of the original kerosene. However, some soils may contain compounds that would be distilled into kerosene and thus influence the refractive-index observation. Therefore, a determination should be made on the kerosene recovered after the steam distillation of each soil, without paradichlorobenzene, and any deviation in refractive index from that of the original kerosene used to compensate for any interfering compounds that may be present. Furthermore, kerosene is not a pure compound, and the refractive indices of different kerosenes are not identical. Consequently, the relation between refractive

index difference and concentration should be established by using any kerosene selected for this purpose.

It is suggested that this method may find application in the determination of other volatile compounds, such as naphthalene or betanaphthol.

#### SUMMARY

A method is presented for the determination of paradichlorobenzene in soil mixtures. The method consists in the removal of paradichlorobenzene from the mixture by steam distillation, its recovery in highly refined kerosene, and the determination of the difference between the refractive index of the solution and that of the kerosene. The relationship between the refractive-index difference and the weight of paradichlorobenzene in soil mixtures may be expressed by a linear regression formula. The method was applied to the determination of paradichlorobenzene in soil mixtures used in the treatment of nursery plants. The mean difference in duplicate analyses was about one-fourth pound of paradichlorobenzene per cubic yard. It is suggested that this method may find application in the determination of other volatile compounds, such as naphthalene or betanaphthol.

#### AIDS FOR DETERMINING CRUDE FIBER

By ERWIN J. BENNE (Agricultural Experiment Station,  
East Lansing, Michigan)

Fiber and crude fiber are terms applied to those constituents of plant materials and feed stuffs that do not dissolve when digested in turn with boiling sulfuric acid and sodium hydroxide solutions of specified concentrations for prescribed periods of time. In its quantitative determination a weighed sample is refluxed with 1.25 per cent sulfuric acid solution for 30 minutes, after which the hot solution is removed by filtration through cloth, which retains the undigested residue. This residue is washed with boiling water, returned to the digestion flask with boiling 1.25 per cent sodium hydroxide solution, and again refluxed for 30 minutes. The material that remains is then collected on the filter cloth, washed free of alkali with hot water, and transferred to a Gooch crucible containing a filter pad of asbestos. After the crucible and its contents are dried and weighed they are ignited, and the weight of the crude fiber is obtained by difference.

Since each step of such an empirical procedure must be executed promptly and accurately if reproducible results are to be obtained, it is obvious that aids that expedite performance of the essential operations are of value. The purpose of this paper is to present two improvised articles that the author and his assistants use to facilitate this determination.



Digestion of the sample is customarily carried out in an Erlenmeyer flask of 750–1000 ml. capacity. It is hazardous to pour boiling acid or alkali from these vessels and to wash the residue from them with a stream of boiling water unless the hands of the manipulator are adequately protected. Gloves made from cotton flannel or other cloth provide fair insula-

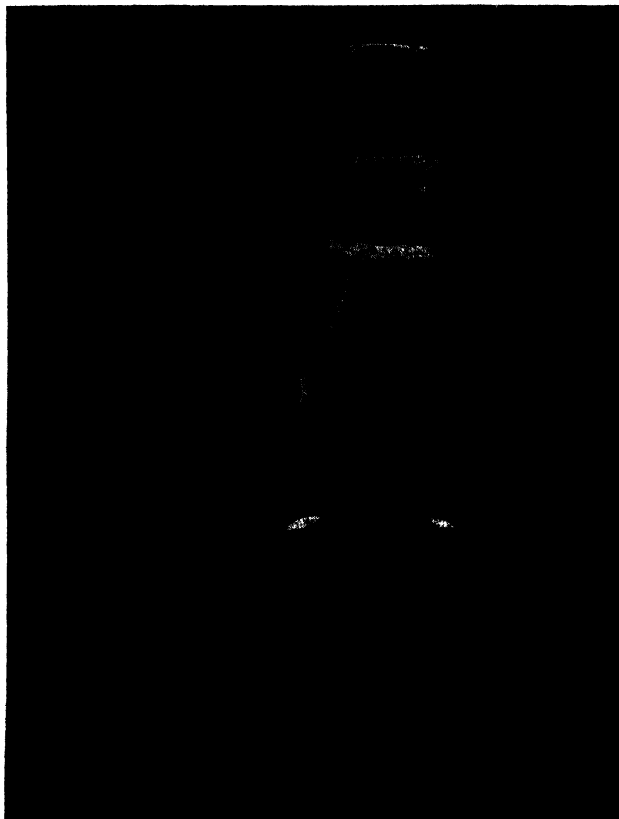


FIG. 1.—THE HANDLE.

tion against heat, but they are cumbersome and not impervious to the boiling liquids. Rubber gloves protect the hands against penetration of the hot liquids, but they are poor heat insulators.

As the writer was unaware of any easily attached clamp designed for handling these flasks during such use, he devised the combined handle and clamp shown in Figure 1. The handle was fashioned from maple wood, and the clamp jaws were obtained from a Cenco universal clamp. The rivet holding the jaws together was removed, and the holes were enlarged to accommodate a No. 6, round-headed, wood screw, which was used to fasten the jaws to the handle as shown.

Figure 2 shows an insulated scoop designed to hold the filter cloth from which the fiber is transferred to the digestion flask with boiling alkali solution and to the Gooch crucible with hot water. The idea of a scoop for this purpose is not original with the writer. Indeed, one made from sheet metal has been used in this laboratory for many years, its disadvantage being that it was not insulated against conductivity of heat. The scoop pictured was made from a piece of sheet iron fashioned to a convenient size and shape. A block of wood was hollowed out until it would accommodate the scoop, which was fastened to the block with screws from the

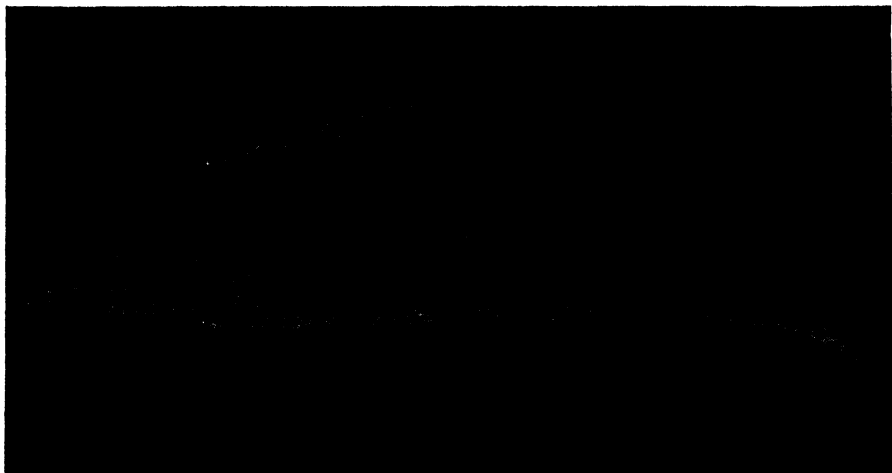


FIG. 2.—THE SCOOP.

inside. The heads of the screws were covered over with solder to make the inner surface of the scoop smooth and prevent the hot liquids from seeping through to the wood. The block was then reduced to a convenient size, its small end was shaped to fit the curve in the neck of the digestion flask, and its sides were grooved to provide finger holds. Plastic wood was used to fill any spaces remaining between the metal and the block, which was finally treated with a penetrating wood preservative. This scoop possesses an additional advantage in that it rests securely on a flat surface and does not tip as the filter cloth is fitted into it.

These articles have proved to be convenient and effective, and it is obvious that the handle would be of value in any work that involves the use of large Erlenmeyer flasks containing hot liquids.

## TOBACCOS CLASSIFIED ACCORDING TO NATURE OF THEIR ALKALOIDS

By L. N. MARKWOOD\* and W. F. BARTHEL (Bureau of Entomology  
and Plant Quarantine, Agricultural Research Administration,  
U. S. Department of Agriculture)

Tobaccos are usually graded commercially according to the physical qualities associated with their use as smoking material, but because the tobacco alkaloids are important as insecticides a classification based upon the nature of these alkaloids is also of interest.

It has been known for many years that nicotine is not the only alkaloid in tobacco, and that it occurs normally in association with a relatively small quantity of other closely related alkaloids.<sup>1</sup> Recently, however, some strains of tobacco have been found to contain nicotine only as a minor alkaloid constituent, while nornicotine is the major alkaloid.<sup>2,3</sup> Since such strains are the obvious source materials of nornicotine, a convenient method of recognizing them seemed to be desirable. The method described here makes possible the ready selection of a nornicotine tobacco from the melting point of the picrate of the total alkaloid component. The writers are not aware of the application of this principle in recognizing the nature of the entire alkaloid complex as distinguished from that of any particular alkaloid. As a result of this study it also seems logical to classify all tobacco under three types—nicotine, nornicotine, and mixed. The first two types are not to be regarded as completely true to designation, but they are predominantly so. The other alkaloids in tobacco may be ignored because of their small quantity.

An important feature of the method presented is the manner of releasing the alkaloids from the tobacco material. The sample is treated with sulfuric acid (9+1), whereby the cellular structure is quickly broken down with rapid release of the organic bases into the acid solution. This treatment appears to leave the alkaloids entirely unchanged, and it is preferable to the older lengthy methods that involve alkalizing the tobacco, extracting with an organic solvent, and the use of a special apparatus. The success of the extraction also depends upon the fineness of the sample.

### METHOD

A 2 gram sample of powdered tobacco, in a small beaker (100 ml.) placed in a moderately cool water bath, is mixed with 6 ml. of  $H_2SO_4$  (9+1) until the tobacco is completely disintegrated (2-3 min.). After reaction has subsided the beaker is removed from the water bath, 50 ml. of water is stirred in without cooling, and the covered beaker is placed on the steam bath for 30 minutes. The mixture is cooled to room temperature and filtered. The filter and contents are washed with water to

\* Now with the Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce.

<sup>1</sup> A. Pictet and A. Rotschy, *Compt. rend.*, 132, 971-2 (1901).

<sup>2</sup> P. Koenig, *Tabak. Allgemeiner und Chemischer Teil*, p. 296. In "Handbuch der Lebensmittel-Chemie," VI Band, Berlin (1934).

<sup>3</sup> L. N. Markwood, *Science*, 92, 204-5 (1940); *This Journal*, 23, 804-10 (1940).

absence of color or to a negative test with silicotungstic acid. The volume of filtrate is held to less than 100 ml.

While being cooled the liquid is made strongly alkaline with solid NaOH and then exhaustively extracted with benzene; five extractions of 30 ml. each usually suffice. The slightly yellow, clear benzene extract, free from any aqueous solution and dried if necessary with NaOH, is extracted with a slight excess (10 ml.) of 0.1 *N* HCl. The benzene is then washed twice with 5–10 ml. of water, and the washings are added to the acid extract. A weak solution of NaOH (0.1 *N*) is added until the liquid is just neutral to phenolphthalein. If a turbidity develops, the liquid is filtered. Sufficient saturated aqueous picric acid solution (10–25 ml.) is added to precipitate all the alkaloids. The liquid is boiled to dissolve the precipitate and cooled slowly. The crystals are filtered off, washed first with dilute picric acid solution and then with water, and dried. The melting point of the crystals is determined as usual in a capillary tube.

#### NOTES ON THE METHOD

A 2 gram sample is usually taken, but if the alkaloid content is high, 1 gram suffices, and the quantity of sulfuric acid is then reduced to 3 ml. Sulfuric acid (9+1) appears to be the ideal strength, since the concentrated acid acts too violently and causes considerable sputtering, while a more dilute acid (such as 8+2) requires a longer period for disintegrating the tobacco. It is believed to be desirable to keep the mixture cool at this stage to avoid possible oxidative effects. The odor of sulfur dioxide is much in evidence during the first few minutes, and it evidently comes from reduction of the sulfuric acid by organic matter. Later an odor suggestive of formic acid, or possibly hydrogen chloride, develops.

Heating on the steam bath is necessary to make the mixture readily filterable. Cooling before filtration helps to make nonalkaloidal matter insoluble. Notwithstanding the formation of solids when the sulfuric acid solution is alkalized, the benzene layer separates readily if the liquid is fully saturated with sodium hydroxide. In melting-point determinations the rate of heating must be controlled, as too rapid heating results in high values. The rate given in the U. S. Pharmacopoeia is recommended, viz., about 0.5 degree per minute when near the melting point.

Characteristic of nornicotine is the colloidal appearance of the picrate as it begins to crystallize from solution, whereas nicotine picrate crystallizes out directly in visibly crystalline form. Nicotine picrate crystallizes in long needles. Nornicotine picrate is usually obtained as short, coarse needles, frequently irregular, and slightly curved, clumped together in T and Y shapes.

The picrate of nicotine melts at 223°–4°C. (corr.) with decomposition; that of nornicotine melts at 191°–2°C.<sup>4</sup> to a clear stable liquid. The picrate of a nicotine type usually has a rather sharp melting point, i.e., within about 3 degrees. Picrates of the mixed and nornicotine types are characterized by a much wider spread, which can be as great as 20 degrees. If the

<sup>4</sup> M. Ehrenstein, *Arch. Pharm.*, 269, 827–59 (1931).

TABLE 1.—*Melting points of the alkaloid picrates of various tobaccos*

DESCRIPTION	SOURCE	YEAR	MELTING POINT °C. (CORR.)
<i>Nicotine Type</i>			
White Burley	Kentucky	1939	218–220
Cigar Binder Leaf	Florida		217.5–220
Connecticut Valley Broadleaf	Connecticut		222–3
Low-nicotine, Texas-Cuban	Virginia	1940	221–3
High-nicotine, Texas-Cuban	Virginia	1940	222–3
Pennsylvania Broadleaf	Pennsylvania	1939	222–3
Havana Seed	Wisconsin	1937	221–3
Maryland Mammoth	California	1936	220–2
Catterton's Maryland Broadleaf	Maryland	1939	220–1
Long Red Maryland Medium Broadleaf	Maryland	1939	220–221.5
White Burley, Kelley	West Virginia	1937	220–223.5
Fire-cured, Madole	Tennessee	1936	221–4
Dark Air-cured	Virginia	1935	220.5–224
Turkish, Xanthi	Virginia	1940	221.5–224
Low-nicotine, German	Virginia	1940	221–2
South America, Ambalema	Virginia	1940	220–4
Russia, Red Flower	Virginia	1935	218–23
Flue-cured, Gold Dollar	South Carolina	1940	219–23
Olson ( <i>Nicotiana rustica</i> )	California	1940	219–20
Winnabago ( <i>Nicotiana rustica</i> )	Virginia	1940	219.5–221.5
Brazilia ( <i>Nicotiana rustica</i> )	Virginia	1940	222–223.5
<i>Mixed Type</i>			
White Burley	Kentucky	1939	192–203.5
White Burley, Halley	Tennessee	1934	198–215
<i>Nornicotine Type</i>			
Robinson's Maryland Medium Broadleaf	Maryland	1938	181.5–186.5
Flue-cured, Cash (Moss 1937)	Virginia	1940	178–190.5

spread is appreciable, the limits may not be determined with precision, and duplicate tests may not agree closely. However, in such cases it is sufficient to establish the over-all picture of the melting point in order to make the proper classification.

#### EXPERIMENTAL RESULTS

Approximately 90 samples of tobacco, including some of *Nicotiana rustica*, were examined by this method. Typical results are shown in Table 1.

Melting points of tobacco alkaloid picrates ranging from 175° to 224°C. have been observed. The finding that the great majority of samples belong to the nicotine type harmonizes with the long-known fact that the characteristic alkaloid of tobacco is nicotine and that any accompanying alkaloids are usually of minor consequence.

The two samples of the mixed variety represent special tobaccos cultivated as "low-nicotine" types. The two tobaccos in the nornicotine group are of special interest because this alkaloid is still rare. The variety known as Robinson's Medium Broadleaf has been investigated,<sup>\*</sup> but Flue-cured, Cash (Moss 1937), has not been investigated further than is reported here. It may prove equally valuable as a source material of nornicotine.

#### ACKNOWLEDGMENT

The writers are indebted to W. W. Garner and Frank B. Wilkinson, of the U. S. Department of Agriculture, for furnishing most of the samples examined.

#### SUMMARY

A method is presented for classifying tobaccos according to the nature of the alkaloids. Powdered tobacco is treated with sulfuric acid (9+1), water is added, and the mixture is warmed, then cooled and filtered. The filtrate is made strongly alkaline and extracted with benzene; the benzene extract is then extracted with 0.1 *N* hydrochloric acid. The alkaloid picrate is formed and its melting point taken. According to the melting point, tobaccos are classified as follows: Nicotine type (m.p. 215°–224°C.), mixed type (m.p. 190°–215°C.), and nornicotine type (m.p. 175°–200°C.). Nearly all of the 90 samples of tobacco tested, including *Nicotiana rustica*, belong to the nicotine type. Two specimens of the nornicotine type are recorded; one, a Maryland tobacco known as Robinson's Medium Broadleaf, was previously known to be of the nornicotine type; the other, Flue-cured, Cash (Moss 1937), is reported for the first time.

### QUANTITATIVE DETERMINATION OF NICOTINE AND NORNICOTINE IN MIXTURES

By L. N. MARKWOOD\* (Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture)

The occurrence of nicotine and nornicotine together in nature makes it desirable to have a method for the quantitative determination of these alkaloids in the presence of each other. P. Koenig<sup>1</sup> in 1934 described such a method based upon dual distillation from (1) magnesium oxide solution, whereby only nicotine was said to distil over, and (2) from sodium hydroxide solution, whereby both alkaloids passed over. More recently C. R. Smith<sup>2</sup> described a method whereby separation was accomplished by fractional steam distillation. This method, like Koenig's, is based upon a

\* Now with the Bureau of Foreign and Domestic Commerce, U. S. Department of Commerce.

<sup>1</sup> Tabak. Allgemeiner und Chemischer Teil, pp. 296–298. In "Handbuch der Lebensmittel-Chemie," VI Band, Berlin (1934).

<sup>2</sup> Ind. Eng. Chem., Ind. Ed., 34, 251 (1942).

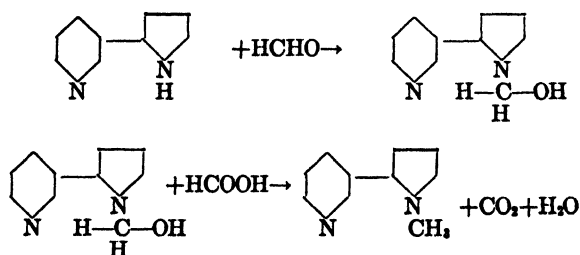
physical separation, and while probably the more accurate it appears to be subject to the operator's skill and patience in conducting the fractionation, which, to be done properly, is time consuming.

The method presented here is based upon chemical reactions. It consists of two parts—(1) treatment with nitrous acid, whereby the nornicotine is converted to the nitroso derivative while the nicotine is unaffected and is steam-distilled away from the nonvolatile nitroso-nornicotine; and (2) methylation of another portion of the sample with formaldehyde and formic acid, whereby the nornicotine is converted to nicotine and the treatment is then as for nicotine alone. The nornicotine is found by difference.

The first part of the method is based upon the well-known difference in behavior of secondary and tertiary bases with nitrous acid. A. A. Shmuk and A. Borozdina<sup>3</sup> utilized this principle in determining nicotine in the presence of anabasine. They effected nitrosation by treatment of the aqueous solution with sulfuric acid and sodium nitrite at 40°–50°C. In the present application a weaker acid, such as acetic or citric, is preferred and treatment is at room temperature (25°–30°C.).

The methylation of nornicotine to nicotine is adopted as the second main step in the analysis, since nornicotine cannot be steam-distilled so readily as nicotine. Späth and Zajic<sup>4</sup> successfully employed methylation via formaldehyde and formic acid on anhydrous nornicotine for the purpose of identification.

It is now established that the methylation can be made quantitative in dilute aqueous solution. The reaction may be illustrated thus:



#### ALKALOID SPECIMENS USED

The nicotine used in the experiments was prepared from commercial 95 per cent nicotine. It was purified for removal of secondary bases by treatment with sulfuric acid and sodium nitrite. The recovered alkaloid was distilled twice. Its specific rotation,  $[\alpha]_{20/D}$ , was  $-168.0^\circ\text{C}$ . The picrate melted at  $224^\circ\text{--}5^\circ\text{C}$ . (cor.). Both these values agree well with those in the literature.

The nornicotine used was isolated from Maryland tobacco (Robinson's Medium Broadleaf).<sup>5</sup> The picrate melted at  $190^\circ\text{--}1^\circ\text{C}$ ., in agreement with

<sup>3</sup> *J. Appl. Chem. (U.S.S.R.)*, 12, 1582–85 (1939); *C. A.*, 34, 7063 (1940).

<sup>4</sup> *Ber.*, 68, 1667–70 (1935).

<sup>5</sup> L. N. Markwood, *This Journal*, 23, 804–10 (1940).

the accepted value.<sup>6</sup> The specific rotation,  $[\alpha]_{25/D}$ , was  $-76.4^{\circ}\text{C}$ . Since the accepted rotation of 1-nornicotine is  $-88.8^{\circ}$ ,<sup>4</sup> this specimen represented a mixture of the *l* and *d, l* isomers in the approximate ratio of 86:14.

A dilute aqueous solution of each alkaloid was prepared and standardized by titration with standard acid (methyl red indicator).

#### NICOTINE IN PRESENCE OF NORNICOTINE

The aqueous alkaloid solution, diluted to ca. 15 ml. in a 300 ml. Kjeldahl flask, was treated with 2 ml. of 30% acetic acid plus 10 ml. of freshly prepared 5%  $\text{NaNO}_2$  solution. The solution was allowed to stand at room temperature for 15–20 minutes. It was then made slightly alkaline to phenolphthalein with  $\text{NaOH}$  (30% at first, followed by 5%). After the neck of the flask had been washed down, 2% acetic acid was added dropwise until the pink color of the phenolphthalein was just dispelled. Then, to remove vapors of both acetic and nitrous acids, which if unremoved would pass into the distillate, the space over the solution was aspirated with a current of air for several seconds, 10 ml. of *pH* 10 buffer solution was added, and the liquid was steam-distilled at low volume (ca. 15 ml.) until all the nicotine had passed over.

The nicotine in the distillate was then determined by silicotungstic acid precipitation or by titration with standard acid. As a matter of convenience it was usually determined by titration with 0.05 *N*  $\text{HCl}$  in the presence of methyl red indicator (0.02%). The usual practice was to collect and titrate the distillate in fractions of 75 ml. each, so that the course of distillation could be followed. Due allowance was made for the deduction of a blank for each fraction after the first. The time required for this part of the analysis was 1.5 hours.

Note: Alkalinity was controlled at *pH* 10 for the distillation, since in highly alkaline solution there is danger of distilling over some of the nitrosamine. At *pH* 12.5 there was obtained a distillate that gave a positive nitroso reaction (Liebermann test) and contained a small quantity of titratable base precipitable with silicotungstic acid.

Experiments were conducted with nicotine alone and with both alkaloids present. The results in Table 1 show that the recovery of nicotine from a solution containing no nornicotine was slightly higher, although perhaps not significantly, when acetic acid was used in conjunction with sodium nitrite than when sulfuric acid was used. The recovery in the acetic acid treatment—practically 98 per cent—was considered satisfactory. The small difference between this recovery and the theoretical may be due to partial destruction of nicotine by nitrous acid, and to the presence in the nicotine of a small quantity of basic impurity.

Tests with silicotungstic acid on the residues left after distillation resulted as follows: Without nitrous acid, negative; sulfuric acid and sodium nitrite, slightly positive; acetic acid and sodium nitrite, negative. These tests are consistent with the view that in the presence of sulfuric acid a secondary reaction occurs, whereby a small quantity of a nonvolatile nitrogenous base is formed. This base is possibly a nitro compound such as is formed by the action of nitric acid on nicotine.<sup>7</sup>

<sup>6</sup> M. Ehrenstein, *Arch. Pharm.*, 269, 627–59 (1931).

<sup>7</sup> H. Lund, *J. Chem. Soc.* (London), 1933, 686–87.



The recovery of nicotine by treatment with acetic acid and sodium nitrite is equally satisfactory in the presence of nornicotine, as shown by results in Table 1.

TABLE 1.—*Recovery of nicotine after various treatments*

TREATMENT	SOLUTIONS		TITER OF 0.05 N HCl	RECOVERY
	NICOTINE	NORNICOTINE		
	ml.	ml.	ml.	per cent
	Nornicotine absent			
Direct titration	10	0	7.60 7.60 Av. 7.60	—
Distillation*	10	0	7.53 7.55 Av. 7.54	100
Sulfuric acid and sodium nitrite	10	0	7.32 7.35 Av. 7.34	97.3
Acetic acid and sodium nitrite	10	0	7.37 7.38 Av. 7.38	97.9
	Nornicotine present			
Direct titration	0	5	4.84	—
Acetic acid and sodium nitrite	0	5	0.03	—
	5	5	3.69	97.9
	10	5	7.44	98.7
	5	10	3.71	98.4

\* The alkaloid was neutralized, 10 ml. of pH 10 buffer solution was added, and the alkaloid was distilled out. A nonvolatile impurity may account for the slightly larger titer by direct titration. Therefore, this treatment was taken as a standard. It is also possible that steam distillation involves a slight destruction of nicotine.

#### EXPERIMENTAL WORK—NORNICOTINE IN PRESENCE OF NICOTINE

Results of the determination of nornicotine via methylation with formaldehyde and formic acid are shown in Table 2. In preliminary experiments the methylation proceeded faster at the boiling point than at lower temperatures, and 15 minutes was sufficient time. Consequently all the treatments (except direct titration) are to be understood as meaning a reflux for 15 minutes.

A comparison of the results by the various treatments shows that the

TABLE 2.—*Determination of nornicotine by various treatments*

TREATMENT OF 5 ML. ALIQUOTS	TITER OF 0.05 N HCl	RECOVERY
	ml.	per cent
Direct titration	4.27	100.0
Formaldehyde and—		
Magnesium oxide	3.51	82.2
Sodium hydroxide	3.97	93.0
	3.94	92.3
	Av. 3.96	92.7
Formaldehyde plus formic acid and—		
Magnesium oxide	3.76	88.2
Sodium hydroxide	4.14	97.0
	4.12	96.7
	Av. 4.13	96.9
Formaldehyde plus formic acid,	4.11	96.1
formaldehyde boiled off,	4.10	96.0
and sodium hydroxide	Av. 4.11	96.1

presence of formic acid is beneficial. Comparison also shows that distillation with sodium hydroxide is preferable to the use of magnesium oxide. The reason for this revolves around the formaldehyde. A considerable excess of formaldehyde was employed to promote a quantitative conversion. Magnesium oxide has no effect on formaldehyde, the excess of which distills over with the nicotine. Formaldehyde, having an acid reaction,<sup>8</sup> diminishes the quantity of standard acid consumed by an amount corresponding to its own titer.

In the treatments in which sodium hydroxide was added for the distillation, an interesting phenomenon was observed. The practice was to add a moderate excess of alkali over that required for the reaction  $2\text{HCHO} + \text{NaOH} \rightarrow \text{CH}_3\text{OH} + \text{HCOONa}$ . The mixture was allowed to stand 15 minutes to allow time for the conversion of the formaldehyde. Soon after the alkaline liquid reached ebullition, the originally colorless solution became first yellow and then orange and remained so to the end of the distillation. This behavior was due to formaldehyde, some of which was still present—possibly because of reversibility of the above reaction—and apparently underwent polymerization.

An experiment was also made to determine whether it was advantageous to eliminate the formaldehyde before distilling off the nicotine.

<sup>8</sup> Julius Schmidt, "A text-book of organic chemistry," English edition by H. G. Rule, p. 178. London, Edinburgh (1936).

Formaldehyde forms with water a minimum-boiling azeotropic mixture that has a concentration of 30 per cent of formaldehyde and boils at 98.7°C.\* The formaldehyde was therefore readily eliminated by boiling the solution; a small quantity of sulfuric acid was included to make sure of retention of the alkaloid. The solution was boiled down with several additions of water until the distillate gave a negative test for formaldehyde (ammoniacal AgNO<sub>3</sub>, with NaOH). The distillation with sodium hydroxide followed. No color formed in this experiment, since the formaldehyde was not present. The recovery by this treatment was not quite so good as when the formaldehyde was not boiled off.

The treatment involving methylation with formaldehyde and formic

TABLE 3.—*Analysis of various alkaloid preparations*  
(Analysis made on 5 ml. aliquots of indicated solutions)

SAMPLE	DESCRIPTION OF ALKALOID	AGE	CONCENTRATION OF TEST SOLUTION	NICOTINE		NORNICOTINE		UNDETERMINED BY DIFFERENCE
			grams/100 ml.	grams	per cent	grams	per cent	per cent
1	Unseparated mixture	13 months	1.3160	0.01073	16.3	0.04551	69.2	14.5
2	Distillate	2 weeks	0.9695	0.04264	88.0	0.00470	9.7	2.3
3	Nornicotine	7 months	1.1568	0	0	0.05328*	92.1	7.9
4	Nornicotine	1 week	1.1304	0	0	0.05439*	96.2	3.8

\* By direct titration.

acid, followed directly by distillation in the presence of sodium hydroxide, gave the maximum recovery—about 97 per cent—and was considered satisfactory for adoption.\*

#### PROCEDURE ADOPTED

A solution containing 5 ml. of aqueous alkaloid solution, 2 drops (0.1 ml.) of formic acid (87%), and 5 ml. of formaldehyde (37%) was gently refluxed 15 minutes in a 300 ml. Kjeldahl flask; a few pumice chips were included to promote smooth boiling. The solution was cooled, and 10 ml. of NaOH solution (300 g./liter) was added. After the solution had stood 15 minutes, it was steam-distilled, and the distillate was titrated as described for the determination of nicotine. The difference between the two titers represented the nornicotine equivalent.

1 ml. of 0.05 N HCl equals 0.0074 gram of nornicotine or 0.0081 gram of nicotine.

This portion of the analysis was conducted in 2 hours.

#### ANALYSES OF VARIOUS ALKALOID PREPARATIONS

Table 3 lists the results obtained on several alkaloid preparations of unknown composition.

\* E. W. Blair and R. Taylor, *J. Soc. Chem. Ind.*, 45, 68T (1926).

\* Ordinary commercial formaldehyde (87% by weight, 40% by volume) was used. One brand had to be rejected because of a high blank titer on distillation from alkaline solution, but the other, having a zero blank, was satisfactory.

Sample 1 was an alkaloid isolated from Maryland tobacco without separation of the two alkaloids. It is seen that this sample contained more than four times as much nornicotine as nicotine. The relatively large percentage of undetermined matter is attributed chiefly to moisture, which this sample had absorbed on standing 13 months in a flask not closed tightly, and also to decomposition products.

Sample 2 was the alkaloid recovered from the steam distillate of a "total-alkaloid" sample of Maryland tobacco. The analysis confirmed the expected composition in that the product consisted largely of nicotine.

Sample 3 was a lot of nornicotine that had been kept 7 months. By the method of isolation it should have had no nicotine, a fact confirmed by analysis. It also had acquired moisture as well as the inevitable decomposition products.

Sample 4 represented a fresher preparation of nornicotine, with consequently less moisture and other impurities. The nornicotine in Samples 3 and 4 was determined by direct titration, since the absence of nicotine had been demonstrated.

The foregoing method is applicable to the analysis of an alkaloid sample containing the mixed alkaloids or to a solution of the mixed alkaloids. If the alkaloids are in an organic solvent, such as benzene, previous extraction with acid and subsequent liberation of the alkaloids by alkali is necessary.

#### SUMMARY

The determination of nicotine and nornicotine in the presence of each other in aqueous solution is accomplished by treatment of two portions of the solution. One treatment consists in adding to the solution sodium nitrite and acetic acid. The nitroso-nornicotine formed is not volatile in steam from a solution buffered at  $pH$  10. The unchanged nicotine is steam-distilled off and determined by acidimetric titration or by precipitation with silicotungstic acid. The other treatment consists in methylating the nornicotine to nicotine by means of formaldehyde and formic acid. The total alkaloid, now nicotine, is distilled off after addition of excess sodium hydroxide and determined as before. The difference between the two values represents the nornicotine. Details were developed for attaining an accuracy of 97–98 per cent in the determined values for each component of the mixture.

## BOOK REVIEWS

**Cellulose Chemistry.** By MARK PLUNGIAN, Ph.D., Chemist, Homasote Company, Trenton, N. J. Foreword by Louis E. Wise. VII+97 pp., 13 figs. 14.5×22 cm. Chemical Publishing Co., Inc., Brooklyn, N. Y., 1943. Price \$2.25.

The author states in the preface that—

This book was designed primarily as a brief and up-to-date introduction to the chemistry of cellulose for non-cellulose chemists. An effort has been made to achieve a comprehensive outline of modern cellulose chemistry through a concentration of pertinent detail. It is hoped that this book will also serve the practicing cellulose chemist who may wish to review the latest developments in this field. Interpretation of reactions, wherever possible, was made on the basis of the modern conceptions of the micellar structure of cellulose.

The author has accomplished his purpose very well indeed. Obviously, in a book of this character, one should not expect to find an exhaustive treatment of the subject. However, the reviewer feels that in certain chapters the author has oversimplified the treatment. This is particularly evident in Chapter III, where such important pulping operations as the sulphite, sulphate, and soda processes are described in about two pages. This minor commission, however, in no way detracts from the value of the book as a whole. The book is well written and seems to contain no serious errors.

The subject matter is presented in the following nine chapters:

- I. Cellulose and Associated Substances.
- II. Microstructure of Cotton and Wood Fibers.
- III. Isolation and Purification of Cellulose.
- IV. General Properties of Cellulose.
- V. Dispersion of Cellulose.
- VI. Derivatives of Cellulose.
- VII. Modified Cellulose.
- VIII. Constitution of Cellulose.
- IX. Micellar Structure of Cellulose.

The last two chapters, in which the author presents, in a clear manner, present knowledge of the constitution and micellar structure of cellulose, are definitely the best ones of the book. These chapters may be studied with profit, even by the cellulose chemist.—MAX PHILLIPS.

**Aids to Analysis of Food & Drugs.** By J. R. NICHOLLS. Sixth revised edition, 424 pages. Bailliere, Tindall & Cox, 7 & 8 Henrietta Street, Covent Garden, W.C., London, England, 1942.

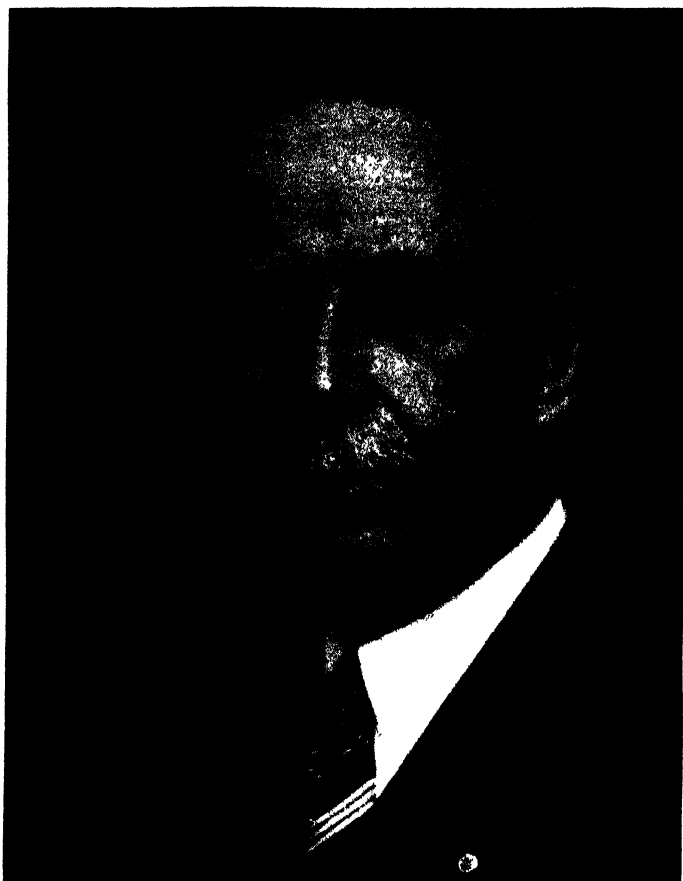
In the sixth revision of this work, the book has retained its 4"×6" pocket size and much of its previous context and style, although the pages have been increased by one-third. Since the last edition the Food and Drugs Act of 1938 of Great Britain has become effective. The work is intended for the use of food and drug chemists. The author is Chemist at the Government Laboratory, London, and the book is written from the point of view of enforcement problems as the food control official knows them—identity, decomposition, and filth. Greatest emphasis is placed on the first named subject. The first edition was offered by T. H. Pearmain and C. G. Moor in 1895. This revision continues the objectives of the original authors—that is, to present aids to the analyst by furnishing him not only directions of procedure but also some bases of interpretation of results. It is in this latter respect that the book differs from other books on methods of analysis of foods and drugs, such as that of the A.O.A.C. and *Schweizerisches Lebensmittelbuch*.

Of the 424 pages, 120 are devoted to dairy products. The remaining pages are

devoted to other foods and to preservatives, coloring matters, poisonous metals, vitamins, drugs, and soaps, with definitions and notes on the British Pharmacopoeia. There is also an appendix containing legal information dealing with English laws on foods.

Obviously the book is expected to find widespread use among the public analysts of England. The data and discussion of composition of foods useful in the interpretation of analyses with references to original articles are based primarily on investigations from English sources. The exceptions to this are so few that they attract attention. For example, in the detection of added water to milk, the Hortvet cryoscopic method is included among the directions. However, the Babcock method for the determination of fat, which is more widely recognized in dairy analysis in this country than the Hortvet method, finds no mention in the book.

This publication would be of interest to American chemists engaged in the analysis of food and drugs, principally because of its discussions on composition and interpretation.—H. A. LEPPER.



DR. ROBERT HARCOURT, 1866-1943

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## ROBERT HARCOURT

Dr. Robert Harcourt of the Ontario Agricultural College, a regular attendant at the annual meetings of the Association of Official Agricultural Chemists from 1901, and President of the Association for the year 1934, died at his home in Guelph, Ontario, March 19, 1943.

Country-born, November 3, 1866, near the town of Goderich, he received his elementary education in rural schools. Graduating from the Ontario Agricultural College in 1893 he became Assistant in the Chemistry Department. In 1901 he succeeded to the Chair of the Department, having in the meantime pursued graduate studies at Harvard University and at the Bureau of Chemistry of the United States Department of Agriculture. In 1908, on a six-months' leave, he visited several of the leading experimental stations of England, the Netherlands, and Germany, studying particularly the organization and work of the chemical laboratories. In 1928 the University of Western Ontario paid tribute to his services to the Province by conferring upon him the honorary degree of D.Sc. In the following year the Government of France granted him the Croix du Chevalier du Mérite Agricole. He retired from the Chair of Chemistry in October 1936, but as Director of the Trent Institute of Baking he retained his connection with the College until 1939, thus completing over forty-five years of service.

Harcourt's work covered all phases of the agricultural chemistry of the time—soils, fertilizers, animal and human foods, sugar beets, agricultural poisons, etc. Results were published in the Bulletins and Annual Reports of the college, in contributions to the agricultural and trade journals, and in addresses to agricultural conventions.

His most important contributions related to wheat flour and to soil surveys. In 1906, following previous studies on the composition and quality of wheat and flour, he established and equipped a milling and flour-testing laboratory. The work of this laboratory during two decades was of such value to the milling and baking industries as to lead to the establishment at the College of a special department, the Trent Institute, with a modern building fully equipped for instruction in baking and for research in cereal chemistry. The topic of Dr. Harcourt's presidential address, published in *This Journal* in 1935, was the history of the wheat-growing industry in Canada and its chemical problems.

Work on the classification and mapping of the soils of southern Ontario, the oldest and still the chief farming district of the Province, was begun in 1914 and has continued to expand until today this Soil Survey commands the services of a number of expert investigators.


Harcourt identified himself with all the Canadian chemical organizations, with the Society of Chemical Industry, with the American Association for the Advancement of Science, of which he was a Fellow, and with the Canadian Society of Technical Agriculturists as a charter and life member. In 1925 he was elected President of the Canadian Institute of Chemistry, an honor indicative of the esteem in which he was held by his professional colleagues. As early as 1901 he associated his Department with the Association of Official Agricultural Chemists.

He took an active part in the affairs of the community in which he lived. He was an Elder of the United Church of Canada and Superintendent of a Mission Sunday School, established by his wife and himself. At various times he served as President of the Canadian Club, the Rotary Club, and the Boy Scouts' Association of Guelph. He also belonged to the Masonic Order.



To keep in touch with the practical aspects of agriculture, Dr. Harcourt owned and operated a farm. His success as a teacher is testified to in the following extract from an obituary written by one of his former students and published in the official organ of the Society of Technical Agriculturists, the *C.S.T.A. Review*. "Thousands of Ontario Agricultural College graduates remember him as an excellent teacher, an upright man, and a good friend. He had an excellent memory for names and faces of former students and charitably forgot the classroom records. He knew the worth of the old institution; he had helped to mould its traditions and he looked to you to carry them on. He reminded you of the old limestone walls, sturdy, dependable, hard and gray, but mellow in the autumn sun of friendship."

J. F. SNELL



## A SOUVENIR OF THE NOTABLE EIGHTH A.O.A.C. CONVENTION OF AUGUST 13-15, 1891

By C. A. BROWNE

Dr. H. B. McDonnell of College Park, Md., has recently sent the writer an autographed menu card containing the signatures of 44 members of the Association of Official Agricultural Chemists and of the Association of American Agricultural Colleges and Experiment Stations who dined together at the Shoreham Hotel in Washington, D. C., Saturday evening, August 15, 1891. Of the 78 members who registered at the Eighth Annual A.O.A.C. Convention, August 13-15, 1891, 48 registered also at the Fifth Annual Convention of the Association of Agricultural Colleges and Experiment Stations, August 12-18, 1891, and 22 signed their names to the menu card. More members of the A.O.A.C. would probably have signed the card had the banquet occurred previous to the termination of its program of reports and papers on Saturday afternoon, August 15, when most of the members left for their homes. The 44 names on the card, with brief biographical data in parentheses, are given below in the order of their signatures:

### *Back of Menu Card*

- H. B. McDonnell\*† (still living; Professor of Agr. Chem. at Md. Agr. College; State Chemist of Md.; President, A.O.A.C.).  
W. Maxwell\* (1854-1931. Asst. Chem., U. S. Dept. Agr.; later Director of sugar-cane experiment stations in Hawaii and Australia).  
Austin Scott† (1848-1922, President of State University of N. J.).  
Wm. McMurtrie (1851-1913, Chief of Div. of Chem., U. S. Dept. Agr.; Chief Chemist, Ill. Agr. Expt. Station; President of Amer. Chem. Soc.).  
F. W. Clarke\* (1847-1931, Chief Chemist, U. S. Geological Survey; President, Amer. Chem. Soc.).  
W. Maxwell, signature repeated by mistake.  
H. H. Harrington\*† (1860-1939, Chemist and later Director, Texas Agr. Expt. Station; President, Texas Agr. College).  
Chas. Richards Dodge (1847-1918, Chief of Fiber Investigations, U. S. Dept. of Agr.).  
E. Lewis Sturtevant (1842-1898, first Director of N. Y. Agr. Expt. Sta.; Agr. investigator, writer and editor).  
H. B. Battle\*† (1862-1929, Chemist and Director, N. Car. Agr. Expt. Sta.).  
B. von Herff\* (?) , representative of German Potash Syndicate).  
I. P. Roberts† (1833-1928, Prof. of Agric., Iowa Agr. College, and later Cornell; leading agr. writer and promoter).  
Wm. Leroy Broun† (1827-1902, President, Ala. Agr. College).  
Thomas F. Hunt† (1862-1927, Prof. of Agron., Cornell; Prof. and Dean, Penn. State and Ohio State; Dean, College of Agr., Univ. of Cal.; Director, Cal. Agr. Expt. Sta.).  
M. A. Scovell\*† (1855-1912, Chemist and Director, Ky. Agr. Expt. Sta.; President, A.O.A.C.).

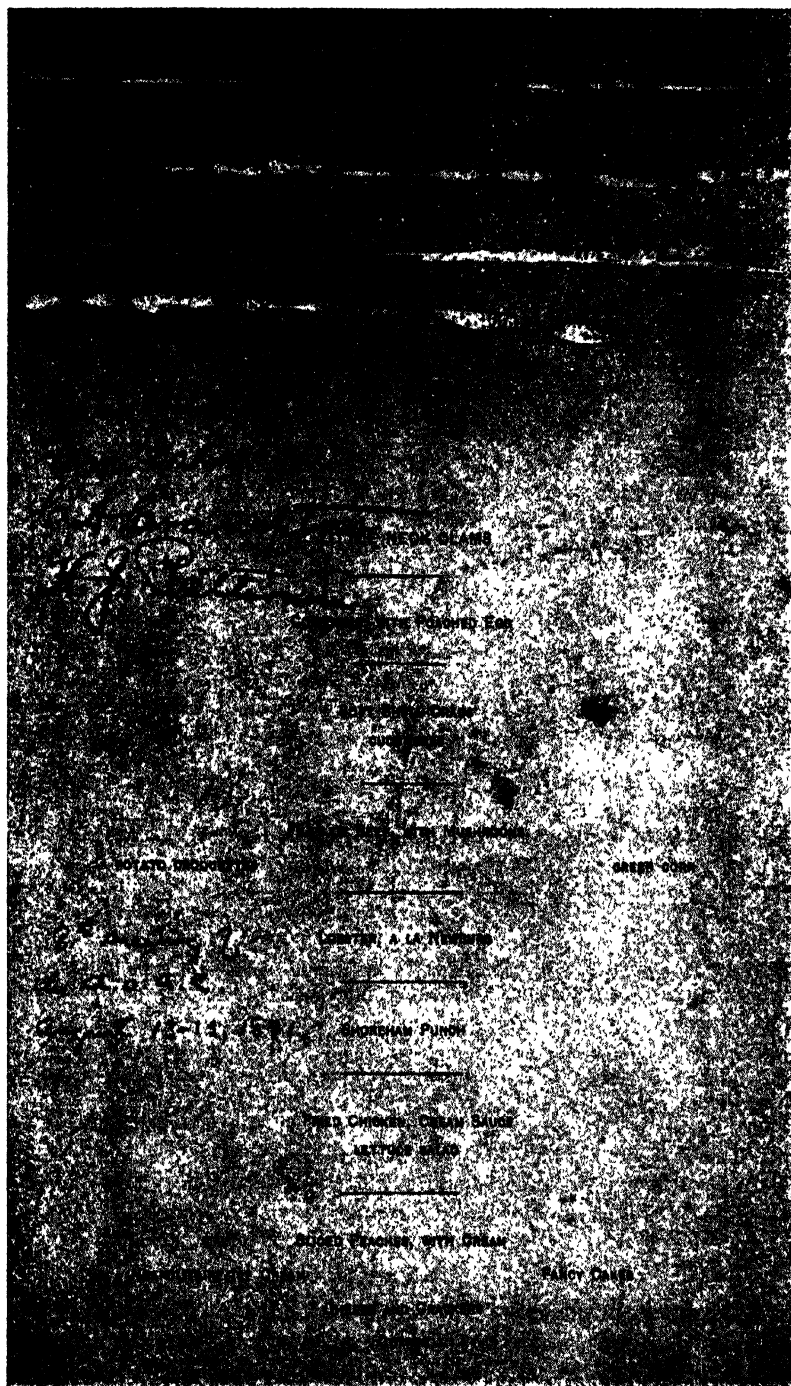
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\* Indicates registration at A.O.A.C. meeting.

† Indicates registration at meeting of Agricultural Colleges and Experiment Stations.

*[The following names are written in cursive script across the page:]*

M. J. ...  
Wm. ...  
H. ...  
J. ...  
R. ...  
S. ...  
T. ...  
U. ...  
V. ...  
W. ...  
X. ...  
Y. ...  
Z. ...



FACE OF MENU CARD

- Chas. E. Thorne† (1846–1936, Director, Ohio Agr. Expt. Sta.).  
 A. W. Harris† (1858–1935, Director, U. S. Office of Expt. Stations; President, Univ. of Maine).  
 A. T. Neale† (1852–1917, Director, Delaware Agr. Expt. Sta.).  
 Hy. E. Alvord† (1844–1904, President, Md. Agr. College).  
 H. W. Wiley\*† (1844–1930, Chief, Bur. of Chemistry, U. S. Dept. Agr.; President, A.O.A.C.; President, Amer. Chem. Soc.).  
 C. V. Riley (1843–1895, Chief Div. of Entomology, U. S. Dept. of Agr.).  
 R. C. Kedzie\*† (1823–1902, Prof. Chemistry, Mich. Agr. College; Chief Chemist, Mich. Agr. Expt. Sta.; President, A.O.A.C.).  
 Edwin Willets\*† (1830–1896, Asst. Secretary, U. S. Dept. Agr.; former President, Mich. Agr. College).  
 Wm. B. Alwood† (still living; retired Prof. of Horticulture, Va. Polytechnic Inst.).  
 N. Robinson\*† (? , Fla. Dept. Agr., Tallahassee).  
 A. de Ghequier† (? , Secretary, National Fertilizer Assoc.).  
 John A. Myers\*† (1853–1901, Chemist and Director, W. Va. Agr. Expt. Sta., President, A.O.A.C.).  
 R. Warington\*† (1838–1907, Chemist, Rothamsted Agr. Expt. Sta., England).  
 Wm. H. Brewer† (1828–1910, Prof. of Agr., Yale Scientific School).  
 Geo. F. Atkinson† (1854–1918, Biologist, Ala. Agr. Expt. Sta.).  
 S. M. Tracy† (1847–1920, Director, Miss. Agr. Expt. Sta.).  
 B. E. Fernow (1851–1923, Chief, Div. of Forestry, U. S. Dept. Agr.).  
 A. J. Cook† (1842–1916, Entomologist, Mich. Agr. Expt. Sta.).  
 A. A. Johnson† (? , President, Univ. of Wyoming).  
 G. S. Fellows\* (? –1901, Prof. of Agr. Chemistry, Md. Agr. College).  
 H. P. Armsby† (1853–1921, Director, Penn. Agr. Expt. Sta.).  
 W. H. Jordan\*† (1851–1931, Prof. of Agriculture and Agr. Chemistry, Penn. State College; Director of Maine Expt. Sta., and of New York Agr. Expt. Sta. at Geneva).  
 S. M. Babcock\*† (1843–1931, Prof. of Agr. Chemistry, Univ. of Wisconsin; Chief Chem., Wis. Agr. Expt. Sta.; President, A.O.A.C.).  
 H. E. Stockbridge† (1857–1930, Director of Indiana Agr. Expt. Sta., and of N. Dakota Agr. Expt. Sta.; President, N. D. Agr. College).  
 G. C. Caldwell\* (1834–1907, Prof. of Agr. Chemistry at Penn. State College and Cornell; Head of Chem. Dept. of Cornell; President, A.O.A.C.; President, Amer. Chem. Soc.).  
 H. A. Huston\*† (still living, Prof. of Agr. Chemistry, Purdue; Director Indiana Agr. Expt. Sta.; State Chem., Ind.; President, A.O.A.C.).

#### *Face of Menu Card*

- A. B. Prescott (1832–1905, Director, chem. laboratories and Dean, School of Pharmacy, Univ. of Mich.; President, Amer. Chem. Soc.).  
 Wm. Frear\*† (1860–1922, Prof. of Agr. Chemistry, Penn. State College; Chief Chemist, Penn. Agr. Expt. Sta.; President, A.O.A.C.).  
 C. A. Crampton\* (1858–1915, Asst. Chem., U. S. Dept. Agr.; Chief, Div. of Chemistry, Inter. Revenue Bur.).  
 H. J. Patterson\*† (still living; Director, Md. Agr. Expt. Sta.; President, Md. Agr. College; President, A.O.A.C.).

The names of Dodge, Fernow, McMurtrie, Prescott, Riley, and Sturtevant on the menu card did not appear among the registrants of the two organizations participating in the banquet. They may have attended the meetings but had failed to register, as often happens, or they may have

been only casual visitors. There were also probably some present at the banquet whom the menu card did not reach. The group of signers was nevertheless a notable one, there being represented upon it 15 men who previously or later were experiment station directors, 6 college presidents, 10 past or future presidents of the A.O.A.C., and 5 past or future presidents of the American Chemical Society. Two of the men, Drs. Wiley and Caldwell, have the unique distinction of having been presidents of both organizations.

The oldest chemist upon the list was Prof. R. C. Kedzie of Michigan Agricultural College, who was born in 1823; the youngest was Dr. H. J. Patterson of Maryland Agricultural College, who was born in 1867. There were two who became nonagenarians, I. P. Roberts who lived to be 95, and C. E. Thorne who reached the age of 90. Of those becoming octogenarians, Dr. Babcock lived to be 88, Dr. Wiley attained 86, Dr. Clarke reached 84, Dr. Brewer 82, and Dr. Jordan 80. Of the four chemists at the banquet still living, Dr. Huston is the oldest, reaching 85 on April 20 of this year, Dr. Alwood attains 84 on August 11, Dr. McDonnell 80 on October 31, and Dr. Patterson 76 on December 17. The oldest living member and former office holder of the A.O.A.C. is Dr. C. W. Dabney, Jr., who was its secretary in 1885 and vice-president in 1886. He was present at the 1891 Convention of Agricultural Colleges and Experiment Stations as President of the University of Tennessee, but his name does not appear on the menu card. He will be 88 on June 19.

A few words should be said about the important contributions of some of the signers of the menu card to the proceedings of the two associations. The most prominent of the scientific events were the six important lectures by Dr. Robert Warington of the Rothamsted Agricultural Experiment Station of England, which were the first to be delivered in the United States under the provisions of the Sir J. B. Lawes Agricultural Trust. These lectures upon various phases of Professor Warington's research work at Rothamsted were afterwards published as Bulletin 8 of the Office of Experiment Stations.

A second important event recorded in the proceedings of the two associations were the reports by Dr. Wiley before the A.O.A.C. (Eighth Proceedings, pp. 170-73) and of Dr. Armsby before the Convention of Agricultural Colleges and Experiment Stations (Fifth Proceedings, p. 74) as chairmen of their committees upon the preparation of exhibits for the World's Columbian Exposition at Chicago in 1893. The Agricultural exhibits later displayed at this exposition had a most important influence in stimulating agricultural chemical research in the United States.

Dr. Caldwell's presidential address before the A.O.A.C. (Eighth Proceedings, pp. 7-13) on the history of efforts for the establishment of uniformity in methods of agricultural and technical chemical analysis was a most thorough presentation and one that can still be read with profit. It is cited as a third important contribution of the 1891 A.O.A.C. meeting.

The very detailed report of Dr. Huston, Referee on Phosphoric Acid (Eighth Proceedings, pp. 81-107) and the equally full report of Dr. Frear, Referee on Nitrogen (Eighth Proceedings, pp. 117-42) with their very complete abstracts of the literature on their subjects, were the two outstanding presentations of the 1891 A.O.A.C. Meeting on analytical methods. Had these and the similar complete reports of other referees in the A.O.A.C. Proceedings of this period been consulted by later writers, they might have avoided certain inaccuracies and at the same time made their list of references more nearly complete.<sup>1</sup>

A report of Dr. Wiley for his Committee on a National Chemical Society, which was presented at the last session of the 1891 A.O.A.C. Convention (Proceedings, pp. 165-69), had much more behind it than appears upon the surface. This report was part of a movement, sponsored chiefly by Drs. Wiley and Clarke and brought first to the attention of the A.O.A.C. at its Sixth Annual Convention in 1889 (Proceedings, pp. 65-68) to organize a National Chemical Society in opposition to the hitherto largely local policies of the American Chemical Society in New York. It was no mere accident that Dr. Wiley fired the bombshell of this report on the Saturday preceding the Third General Meeting of the American Chemical Society in Washington on the following Monday and Tuesday. At this meeting on Monday, August 17, conferees of ten different chemical societies (representing approximately 1,000 American chemists) adopted unanimously the plan outlined in Dr. Wiley's report and the American Chemical Society was then and there reorganized for the first time on a broad national basis. As a result of his part in the movement<sup>2</sup> Dr. Wiley was elected President of the American Chemical Society for the years 1892 and 1893.

As viewed now in its historical retrospect, Dr. Wiley's report on a National Chemical Society was the most important subject of national chemical interest to be presented before the 1891 meeting of the A.O.A.C. It was undoubtedly one of the chief subjects of table conversation among the chemists who took part in the 1891 dinner, of which Dr. McDonnell's autographed menu card remains as an interesting historic souvenir. The A.O.A.C. meeting of 1891, occurring as it did in the midst of meetings of the American Association of Agricultural Colleges and Experiment Stations, of the American Association for the Advancement of Science, of the American Chemical Society, and of the Society for the Promotion of Agricultural Science, was one of the most important in the history of the organization.

<sup>1</sup> In "A Review of the Kjeldahl Determination of Organic Nitrogen" (*Chemical Reviews*, 27, Oct. 1940, pp. 331-350) no mention, for example, is made of the important research work of A.O.A.C. referees on this subject. In this article the use of salicylic acid in the determination of nitro nitrogen is credited to Cope in 1916 instead of to Scovell of the A.O.A.C. in 1887. The writer mentioned this and other errors in the article to the Editor of *Chemical Reviews*, who issued an Errata Sheet of corrections for pp. 343 and 347.

<sup>2</sup> The story of the movement is told in greater detail in the writer's article on "The Chemical Society of Washington and its part in the reorganization of the American Chemical Society," *J. Wash. Acad. Sciences*, 28, May 15, 1938, pp. 233-246.

## REPORT ON BEER

By HUGO W. ROHDE (Jos. Schlitz Brewing Company, Milwaukee, Wis.), *Associate Referee*

Following the suggestion of the Referee, the Associate Referee collaborated with the same group that worked in 1941. The determinations were limited to the following:

- (a) Total acidity by the potentiometric titration
- (b) Sodium chloride
- (c) Dextrin
- (d) Sulfur dioxide

Shortly after the summer meeting of the American Society of Brewing Chemists in 1942, Fred P. Siebel, Jr., President, appointed a number of committees to investigate methods for determining the ingredients of brewing materials and beers. According to the Brewing Chemists' News Letter of September, 1942, Kurt Becker, Siebel Institute of Technology, Chicago, was appointed chairman of the committee for studying methods for the determination of acidity and *pH* of beers. Four members comprise this committee. It would seem to be a duplication to request analysts of the brewing industry to work on the same subject.

According to one of the A.O.A.C. recommendations, the method for the determination of chlorides in beer is to be made official (first action). Hence, for the time being, nothing need be done about this.

The method for the determination of dextrins, *Methods of Analysis*, A.O.A.C., 1940, was found to be satisfactory, but the sample requires a greater dilution, and collaborators have done so of their own accord. The results obtained were quite satisfactory, considering that this determination is made infrequently. Since this work was done, the Associate Referee has conferred with Philip P. Gray, Wallerstein Laboratories, New York, and it was agreed to have George F. Peckham, Jr., Clinton, Iowa, take charge of this work. Mr. Peckham is chairman of the committee for dextrose and dextrin determinations of the Corn Industries Research Foundation, and is well qualified to arrange studies on dextrin determinations. As the firm with which the Associate Referee is connected does not use sugars or sirups in brewing, the laboratory assistants have had no experience with this type of determination. Their chief interest is in the amount of fermentable and unfermentable extract obtained with the particular yeast used in the plant.

It is regretted that the determination of sulfur dioxide was not satisfactory in the last series of collaborative work. Although operators were requested to follow the method described in detail, the results obtained varied considerably. L. V. Taylor, American Can Company, has had considerable experience along the lines of this particular determination, and he may be able to make helpful suggestions.



The subject of Metals in Beer is in the hands of L. E. Clifcorn, Continental Can Company, Chicago. When metal cans lined with a resinous material first came into use for packaging beer, much trouble was experienced, as often the taste was impaired, and imperfect linings exposed the beer to metallic contact, affecting both appearance and taste. These disturbances have been remedied. Infrequent disturbances arise when the cans are not properly prepared in the factory.

## REPORT ON MALT

By CHRISTIAN RASK (Albert Schwill & Co., Chicago, Ill.), *Associate Referee*

This report presents for adoption methods for the determination of moisture, extract, and color in caramel and black malts.

The methods have been tested by collaborative analysis over a number of years and found to give satisfactory agreement. It will be noted that they closely follow the methods already adopted for the analysis of malt and malt adjuncts, materials closely related to caramel and black malts.

The references are to *Methods of Analysis*, A.O.A.C., 1940, XIV.

### CARAMEL MALT

#### EXTRACT

For grinding of sample use a mill for fine grinding as directed under 43.

Weigh ca. 25.5 grams of caramel malt, grind, and adjust to 25 grams ( $\pm 0.05$  gram) by removing excess.

Weigh ca. 25.5 grams of malt of known moisture, extract, and color and having a diastatic power of 100° L. or over; grind, and adjust to 25 grams ( $\pm 0.05$  gram).

Transfer quantitatively the two portions to a mash beaker, mash, and determine specific gravity as directed under 45.

Determine moisture as directed under 40 (b).

Calculate yield by the following formulas:

$$P \times (800 + M \text{ in 50 grams malt} + M \text{ in 50 grams caramel malt})$$

$$\text{Total Extract} = \frac{\quad}{100 - P},$$

where P = grams of extract in 100 grams of wort (Plato), and

M = moisture in grams.

$$(\text{Total Extract} - \text{Extract in 50 grams of malt}) \times 100$$

$$\text{Extract in Caramel Malt} = \frac{\quad}{50}.$$

#### COLOR

Use the mixed wort obtained for extract determination, diluting the wort sufficiently to make the color reading ca. 4.0° L.

Determine color on the diluted wort in a  $\frac{1}{2}$  inch cell with a Lovibond tintometer as directed under 46.

Calculate color by the following formula:

Color of caramel malt =  $2[L \times (D + 1)]$  - color of malt used for conversion,

where L = Color reading on diluted wort, and

D = Parts of water used to dilute one part of wort.

Report the dilution used for making the color reading.

## BLACK MALT

## MOISTURE

Proceed as directed under 40 (b).

## COLOR

For grinding of sample use a mill for fine grinding as directed under 43. As a precautionary measure grind a small quantity of the sample to be analyzed and clean out the mill. For the determination weigh 5.5 grams, grind, and collect all particles by careful brushing of the mill.

Weigh 5.00 grams on an analytical balance, transfer to a glass beaker, add 400 ml. of distilled water at room temperature, and heat to boiling. Boil gently for exactly 5 minutes, cool to room temperature, and without delay transfer to a 500 ml. volumetric flask; make up to volume with distilled water, mix, and filter. Pipet 10 ml. of the filtrate into a 100 ml. volumetric flask, make up to volume with distilled water, and mix.

Determine color of the diluted filtrate in a  $\frac{1}{2}$ -inch cell, using a Lovibond tintometer as specified under 46. Calculate the color found for this filtrate to the same concentration of materials as is used for the regular malt mash (12.5 grams of malt to 100 ml. of water) by the formula:

Color of black malt =  $L \times 10 \times 12.5$ ,

where  $L$  = color reading on the diluted filtrate.

Report color to the nearest whole number.

## REPORT ON pH IN DISTILLED ALCOHOLIC BEVERAGES

By M. ROSENBLATT (Schenley Research Institute, Inc., Lawrenceburg, Ind.), *Associate Referee*

In accordance with the recommendations of the Association (*This Journal*, 25, 70), collaborative work was initiated in 1942. A preliminary investigation concerning the factors affecting the pH determination in alcoholic beverages was reported in 1942 by Liebmann and Rosenblatt (*Ibid.*, 25, 163).

Eleven laboratories expressed a willingness to collaborate, and a set of four samples was sent to each laboratory with the request to determine the pH of the samples electrometrically. The samples consisted of the four distinct types of alcoholic beverages shown in Table 1.

TABLE 1.—Description of samples

CODE	TYPE	ALCOHOLIC CONTENT
		% BY VOLUME
A	Straight Whiskey	43
B	Spirit Blend	43
C	Blend of Straight Whiskeys	45
D	Gin	50

Five different glass electrode electrometers were used by the collaborators in obtaining the data: Coleman Universal Spectrophotometer Model 11, Coleman pH Meter Model 3C, Coleman Industrial pH Meter, Leeds & Northrup Industrial Model Potentiometer, and Beckman pH Meter.

TABLE 2.—*Results of collaborative study on pH*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D	INSTRUMENT
1	4.15	4.23	4.13	6.65	Coleman Universal Spectrophotometer Model 11
	4.15	4.25	4.12	6.68	
	4.15	4.23	4.12	6.68	
	4.16	4.22	4.13	6.70	
2	4.03	4.09	4.02	6.47	Coleman <i>pH</i> Meter Model 3C
	3.95	4.00	3.93	6.56	
	3.99	4.08	3.98	6.05	
	3.99	4.07	3.99	6.64	
	3.96	4.01	3.92	6.10	
	3.96	4.00	3.94	5.50	
	3.93	4.04	3.93	6.35	
3	4.14	4.21	4.14	6.55	Coleman <i>pH</i> Meter Model 3C
4	3.93	4.00	3.89	6.42	Coleman <i>pH</i> Meter Model 3C
	3.94	3.97	3.92	6.41	
	3.96	4.05	3.98	6.40	
5	4.00	4.07	3.97	—	Coleman <i>pH</i> Meter Model 3C
6	3.97	4.03	3.99	6.53	Coleman <i>pH</i> Meter Model 3C
7	4.02	4.25	4.15	5.00	Coleman Industrial <i>pH</i> Meter
	4.05	4.22	4.01	5.34	
	4.02	4.24	4.17	5.42	
8	3.82	3.90	3.82	5.60	Leeds and Northrup Coleman Industrial Beckman <i>pH</i> Meters
	3.78	3.80	3.82	5.52	
	3.84	3.89	3.82	6.17	
9	3.97	4.01	3.97	6.10	Beckman <i>pH</i> Meter
	3.96	4.01	3.96	6.63	
	3.96	4.01	3.96	6.65	
	3.98	4.10	3.97	—	
	3.98	4.10	3.96	—	
	3.98	4.10	3.97	—	
	—	4.10	—	—	
	—	4.03	—	—	
	—	4.03	—	—	
10	4.00	4.09	4.00	6.44	Coleman <i>pH</i> Meter Model 3C
	3.99	4.08	4.00	6.43	
	3.99	4.08	3.99	6.44	
	—	—	—	6.60	
	—	—	—	6.60	
11	3.95	4.04	3.96	6.88	Leeds and Northrup Industrial Model Potentiometer
	3.97	4.06	3.98	6.95	
	3.98	4.12	3.98	6.90	
	—	—	—	6.87	
	—	—	—	6.67	
	—	—	—	6.85	
Average	3.99	4.07	3.99	6.35	
Standard Deviation	0.05	0.08	0.07	0.38	

Each collaborator was requested to follow the technic recommended by the manufacturer of the instrument.

#### COLLABORATORS

The Associate Referee appreciates the cooperation of the following collaborators:

1. C. S. Ferguson, Dept. of Public Health, Boston, Mass.
2. M. Rosenblatt
3. J. Banks, Geo. T. Staggs Co., Frankfort, Ky.
4. J. Harris, Old Quaker Co., Lawrenceburg, Ind.
5. H. Levin, Oldetyme Distillers, Jersey City, N. J.
6. H. Hartman, Jos. S. Finch & Co., Schenley, Pa.
7. N. M. Erb, U. S. Industrial Chem., Baltimore, Md.
8. I. R. Sipherd, National Dist. Prod. Corp., New York, N. Y.
9. C. T. Carson, Frankfort Dist. Inc., Baltimore, Md.
10. A. Herman, Jos. E. Seagrams & Sons, Louisville, Ky.
11. P. Valaer, Alcohol Tax Unit, Washington, D. C.

#### DISCUSSION OF RESULTS

The data reported by the collaborators, together with the calculated averages and standard deviations for each sample, are shown in Table 2.

Evidently the determination of the *pH* of an unbuffered liquid such as gin (Sample 4) cannot be determined by the methods used in this collaborative study without further refinement, since the standard deviation in this case ( $\sigma=0.38$ ) is well beyond the differences to be measured. Additional collaborative work on the *pH* determination of such liquids as gin and neutral spirits is planned. The discussion excludes Sample 4.

The standard deviation of the *pH* determination of whiskey is in the neighborhood of 0.07, a result which confirms the earlier preliminary work by Liebmann and Rosenblatt (*loc. cit.*). This indicates that the method in its present form is capable of yielding results on any given sample which should not spread more than about  $\pm 0.1$  *pH* unit. A maximum spread of  $\pm 0.1$  unit can be considered satisfactory since any acidic abnormality in whiskey will generally result in a much greater change in *pH*.

It is to be noted, however, that several of the collaborative results deviated significantly and consistently from the mean. Such regularity in deviation indicates a determinate error in the method. In order to exhibit these deviations more clearly, Table 3 expresses the deviations of each collaborator for Samples 1, 2, 3 in terms of the standard deviation  $(\bar{x}-x'/\sigma)$ .

If  $2\sigma$  is used as a criterion for possible rejection of data (i.e. if  $\bar{x}-x'/\sigma > 2$ , then the result should be considered for rejection), it is clear that the results of Collaborators 1, 3, 8 and possibly 7 fall into this category of possible rejection. Furthermore, each of these collaborative results was always on the same side of the mean. Finally, in the case of Collaborator

8, three completely different instruments were used and the results were always low to such an extent that  $\bar{x} - x'/\sigma > 2$ .

The Associate Referee considers that these deviations were caused by undetected variation in the pH value of the standard buffer solution used to check the instrument. It is unfortunate that the collaborators were not requested to give more complete details covering this point, but the avail-

TABLE 3.—*Deviations from the mean*

COLLABORATOR	$\frac{\bar{x} - x'}{\sigma}$		
	SAMPLE A	SAMPLE B	SAMPLE C
1	3.2	2.0	2.0
2	0.4	0.4	0.4
3	3.0	1.7	2.1
4	1.0	0.7	0.9
5	0.2	0.0	0.3
6	0.4	0.5	0.0
7	0.8	2.1	1.7
8	3.6	2.6	2.4
9	0.4	0.2	0.3
10	0.0	0.1	0.1
11	0.4	0.0	0.3

$\bar{x}$  = Overall mean

$x'$  = Collaborator's mean

$\sigma$  = Overall standard deviation

able information shows that different standard buffer solutions were used in different laboratories. Some laboratories used buffers purchased from the manufacturer of the instrument, whereas others used buffers prepared in their own laboratories. The laboratory of the Associate Referee has encountered considerable difficulty with prepared concentrated buffers supplied by the manufacturer.

That the use of a reliable standard buffer should minimize the variation in the method and reduce the magnitude of the standard deviation is confirmed by the results from any one laboratory where only one buffer solution was used. Table 4 shows the standard deviations (based on the individual collaborator's average) for Collaborators 2, 8, and 9.

TABLE 4.—*Individual standard deviations on pH*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C
2	0.03	0.03	0.03
8	0.02	0.04	0.00
9	0.01	0.04	0.01

These deviations are less than one-half of the general over-all standard deviations.

## REPORT ON BAKED PRODUCTS OTHER THAN BREAD

By NILES H. WALKER (National Biscuit Company, New York,  
N. Y. ), *Associate Referee*

Biscuits and crackers are not specifically mentioned in *Methods of Analysis, A.O.A.C.*, 1940, but in Chapter XX, page 234, under the heading "Baked Products Other Than Bread—Tentative (not containing fruit)", the methods referred to are suitable for most of the determinations required in the analysis of this class of baked goods.

These methods have been found to give satisfactory results for determinations of total solids, ash, and protein contents of baked products other than bread not containing fruit (Voris, *This Journal*, 23, 537, and 24, 627). It was recommended that further study of the methods for determining crude fiber, fat by the acid hydrolysis method, and moisture in baked products other than bread, containing fruit, be continued.

With the consent of the Referee, the Associate Referee has confined the work covered by this report to the determination of moisture content and of total fat content by the acid hydrolysis method of two varieties of baked products, other than bread, which contain fruit.

The baked products chosen for this work were: I. Fig Bars and II. Raisin-filled Crackers. These types of crackers have presented the most difficulties from the standpoint of obtaining reasonable checks on analysis. In the first place it is necessary to take a fairly large sample because of the variations in proportion of base cake and fruit in individual pieces. The sample must then be ground and mixed thoroughly until the small charge required for the determination is representative of the product.

The determination of the moisture content of fruits and of products that contain fruit presents a real analytical problem. It is well known that fruit sugars and possibly other fruit constituents decompose, and on drying lose weight other than true moisture. At temperatures higher than 70°C. this loss in weight is appreciably greater than that of the actual moisture content when fairly constant dry weights are obtained.

Fig bars and raisin-filled crackers contain large percentages of fruits that are high in fruit sugar. Naturally, the methods most applicable for determining their moisture content are those that have been found to be most satisfactory for determining the moisture content of fruits and fruit products and sugar products of a semi-solid or sirupy consistency, containing reducing sugars or other constituents that decompose under drying conditions. These methods are listed in *Methods of Analysis, A.O.A.C.*, 1940, Chapter XXVI, 4 and 5, and Chapter XXXIV, 5.

Approximately 4 gram charges of prepared samples of fig bars (Sample IA) and raisin-filled crackers (Sample IIA) were weighed out and treated precisely as directed in the A.O.A.C. method, Chapter XXXIV, 5, to the point where oven drying begins. They were then dried under different

oven temperatures and conditions to show the variations in percentage loss in weight.

The method studied for determining the fat content of these samples is referred to in *Methods of Analysis, A.O.A.C.*, 1940, Chapter XX, 11. It was found that it is advisable to modify the method slightly in order to obtain the correct fat contents of these types of crackers.

TABLE 1.—*Moisture (per cent)*

HRS. IN OVEN	70°C. VACUUM OVEN		80°C. VACUUM OVEN		100°C. AIR OVEN		130°C. AIR OVEN	
	I A	II A	I A	II A	I A	II A	I A	II A
4	—	—	16.76	9.77	16.54	10.25	19.44	16.35
6	16.09	9.50	16.88	9.91	17.03	10.92	20.50	17.95
8	16.29	9.65	16.85	9.89	17.20	11.29	21.51	19.20
10	16.40	9.70	16.86	9.88	17.15	11.52	22.17	19.84
12	16.54	9.78	16.93	9.94	17.30	11.84	22.90	20.90
14	16.66	9.83	16.90	9.93	17.44	12.08	23.30	21.29
16	16.66	9.82	16.90	9.96	17.50	12.26	23.80	21.83
18	16.68	9.84	16.90	9.98	17.60	12.51	24.22	22.31
20	16.66	9.84	16.96	10.05	17.63	12.74	24.70	22.68
22	16.68	9.84	16.99	10.14	17.71	12.83	24.96	23.05
24	16.68	9.84	17.00	10.16	17.88	13.11	25.58	23.34
40	16.69	9.86	17.20	10.44	—	—	—	—

The procedure in detail is as follows:

Weigh accurately a charge of ca. 2 grams of the prepared, well-mixed sample and transfer to a Mojonnier tube. Add 2 ml. of 95% alcohol to wet the charge. Add 10 ml. of HCl (25+11). Place the tube in a water bath held at 70°–80°C. and shake at frequent intervals until the charge is thoroughly disintegrated (40–80 minutes, depending on the consistency of the sample and extent of shaking).

If the weighed charge cannot be transferred to the tube before it is digested because of its consistency, or if a Röhrig tube is used, make the digestion in a 50 ml. beaker. Transfer the digested mixture to the tube by draining from the lip of the beaker down a small stirring rod as carefully and completely as possible. Rinse the beaker thoroughly with 10 ml. of 95% ethyl alcohol, transfer to the extraction tube, mix thoroughly, and cool. Rinse the beaker thoroughly with portions of the first 25 ml. of ethyl ether as the ether is added for the first extraction. Repeat the rinsing with portions of petroleum benzin as it is added for the first extraction. Rinse very thoroughly so that all fat is transferred to the extraction tube.

At the end of the digestion period all particles should be completely disintegrated with the exception of hard seeds (in case of fig fillers), and strong fibers. A very small quantity of fat may be retained by seeds after the digestion, but for practical purposes in the analysis of biscuits and crackers containing fruit this is negligible. There may also be very small quantities of fat retained in some fibrous material, but if the sample is thoroughly ground and the digestion carried out as directed, these will be so small that they will be well within the tolerance of experimental error.

When the digestion is made in the extraction tube, at the end of the digestion period add 10 ml. of 95% ethyl alcohol to the digested charge and cool. (The level of the liquids should be in the neck of the Mojonnier tube just below the pouring-off level or just below the drawing-off spigot of the Röhrig tube.) Add 25 ml. of ethyl ether, place tight-fitting stopper in the tube, and shake thoroughly for ca. a

minute. (It was found to be advisable to use a thoroughly cleaned rubber stopper for the Mojonnier tube. There is always danger of fat being retained in a cork stopper. The fitted ground-glass stopper is satisfactory in the Röhrig tube.) Release the pressure very carefully after the tube is shaken so that none of the solvent containing fat is lost. Wash adhering solvent and fat from the stopper back into the extraction tube with a few ml. of the petroleum benzin. (A wash bottle producing a fine jet is convenient for this purpose.) Allow the mixture to stand for a few minutes, then add 25 ml. of petroleum benzin, stopper the tube tightly, and shake thoroughly again for ca. a minute. Release the pressure carefully, remove the stopper, and again wash the adhering solvent and fat back into the tube with a few ml. of petroleum benzin. (If trouble is experienced in releasing the pressure after shaking the tube, cool the tube slightly by holding it under a stream of cold water before removing the stopper.) Allow the mixture to stand until the ether layer is practically clear (10–20 minutes). Pour off as much as possible of the clear ether-fat solution through a small, fast filter by tilting the Mojonnier tube gradually or drawing off through the spigot of the Röhrig tube. (A plug of ether-extracted cotton packed just firmly enough in the stem of a funnel to allow free passage of ether makes an excellent filter for the extractions.) Catch the ether-fat solutions from the extractions in a 250 ml. clean glass beaker. Re-extract the liquid remaining in the tube three times more as directed for the first extraction, each time using 15 ml. portions of the ethers. Each time after the ether-fat solution is drawn off wash off the mouth of the Mojonnier tube or the spigot of the Röhrig tube with a jet of petroleum benzin and drain this ether through the funnel into the beaker.

Evaporate the combined ethers from the extractions from the beaker by fanning or suction. After the ethers are practically off, heat the beaker for ca. 10 minutes on a hot water or steam bath to drive off most of the alcohol and water that has been carried over during the extractions. (The heating on the water bath is to prevent spattering.) Transfer the beaker to a 100°C. air oven, dry for 1 hour, remove, and allow to cool. Re-dissolve the dried fat in 15–20 ml. of a mixture of equal parts of ethyl ether and petroleum benzin and filter through a small fat-free filter paper into a beaker that has been previously dried in the 100°C. air oven, cooled in a desiccator, and weighed. (Aluminum beakers have been found to be very satisfactory for weighing the purified fat as they are light in weight and cool to room temperature rapidly.) Wash all traces of fat from the glass beaker, filter paper, and funnel into the tared beaker with a stream of petroleum benzin from a wash bottle. Evaporate ethers from the tared beaker by fanning or suction and dry the purified fat for ca. an hour in the 100°C. air oven, cool in the desiccator, and weigh as soon as it attains room temperature.

The moisture and fat content of prepared samples of fig bars (Sample IA) and raisin-filled crackers (Sample IIA) were determined by three analysts in this laboratory. The procedure described on page 485 of *Methods of Analysis, A.O.A.C.*, 1940, was followed for moisture determinations, but with an increase in drying time to 16 continuous hours in the oven. The modified acid hydrolysis procedure described previously was followed for the determination of fat. These results are listed in Table 2.

Two batches of these types of crackers were ground twice through a food chopper and mixed thoroughly. Several sets of prepared samples from the batches were then sealed in tightly capped sample bottles and delivered to analysts for determinations of the moisture and fat contents. The results obtained by the collaborators are listed in Table 3.



TABLE 2.—*Fat (per cent)*

COLLABORATOR	MOISTURE—16 HOURS AT 70°C. IN VACUUM OVEN		FAT—MODIFIED ACID HYDROLYSIS METHOD	
	SAMPLE IA	SAMPLE IIA	SAMPLE IA	SAMPLE IIA
A	16.63	9.85	5.65	5.81
	16.65	9.85	5.63	5.81
	Av. 16.64	9.85	Av. 5.64	5.81
B	16.60	9.81	5.62	5.62
	16.42	9.79	5.56	5.60
	Av. 16.51	Av. 9.80	Av. 5.59	5.72
				5.69 Av. 5.66
C	16.67	9.88	5.63	5.80
	16.68	9.84	5.62	5.86
	Av. 16.68	Av. 9.86	Av. 5.63	Av. 5.83

TABLE 3.—*Collaborative results on fat and moisture in crackers (per cent)*

COLLABORATOR	MOISTURE—70°C. VACUUM OVEN		FAT—ACID HYDROLYSIS FAT REPURIFIED	
	SAMPLE I FIG BARS	SAMPLE II RAISIN FRUIT	SAMPLE I FIG BARS	SAMPLE II RAISIN FRUIT
A	15.97	9.77	6.27	6.15
	15.91	9.70	6.35	6.18
	Av. 15.94	Av. 9.74	Av. 6.31	Av. 6.17
B	15.92	9.69	6.30	6.10
	15.95	9.70	6.31	6.05
	Av. 15.94	Av. 9.70	Av. 6.31	Av. 6.08
C	15.94	9.77	6.27	6.06
	15.95	9.74	6.29	6.07
	Av. 15.95	Av. 9.76	Av. 6.28	Av. 6.07
D	15.90	10.36	6.22	6.14
	15.86	10.28	6.23	6.12
	Av. 15.88	Av. 10.32	Av. 6.23	Av. 6.13
E	16.00	9.69	6.11*	5.93*
	16.00	9.72	6.18	6.09
	Av. 16.00	Av. 9.71	Av. 6.15	Av. 6.01
F	15.00†	8.98†	5.86	6.28
	15.19	9.00	6.32	6.04
	Av. 15.10	Av. 8.99	Av. 6.09	Av. 6.16

\* Only three extractions made.

† Samples from sample jars reground and probably lost moisture.

## DISCUSSION

Most of the difficulties in determining the moisture content of dried fruits and sirups containing large percentages of reducing sugars are encountered in determining the moisture contents of these types of crackers. Slightly more than 50 per cent of the weight of the raisin-filled crackers is raisins. In addition to the fruit, both types contain appreciable quantities of corn sirup and invert sugar sirups. The consistencies of these types of samples are such that it is necessary to mix them thoroughly with some type of dispersing material that will prevent them from crusting over and retaining moisture. The Associate Referee has found the method recommended on page 485, *Methods of Analysis*, A.O.A.C., 1940, for drying sirups and semi-liquid sugar products on 40–60-mesh quartz sand at 70°C. under a pressure not more than 50 mm. of mercury, to be most satisfactory in obtaining checks and arriving at a point after which there is practically no more loss in weight.

A weighed charge of 3–5 grams should be taken. It is advisable to weigh at least three grams in order to insure a charge that is representative of the sample. If the charge is much more than 5 grams, it is too large for the dish and amount of sand specified by the procedure. In order to mix the samples with the dispersing material, 5–10 ml. of water is required. The excess water should be removed from the dish by heating it on a steam or hot water bath. This operation should be carefully carried out according to directions.

Stirring should be almost continuous to prevent local over-heating, and evaporation of water should never be carried beyond a point where the contents of the dish are of a pasty consistency. Slight overheating on a steam or boiling water bath may raise the final results appreciably.

The results obtained by collaborators on fat determinations by the acid hydrolysis method show fairly satisfactory agreement, considering the physical consistency of these samples, and the difficulties encountered in weighing out and digesting the charges.

It was found advisable to lengthen the digestion period called for in the methods to insure complete disintegration of the charges. The Associate Referee has found no consistent variations in the final results obtained on extractions made from 2 gram charges that were digested at 80°C. for varying lengths of time up to 3 hours, provided the digested charges were thoroughly disintegrated and the extracted fat was free from non-fat substances.

Considerable material other than fat is carried over with the ethers as they are decanted, or drawn off, from the tubes containing the acid hydrolyzed charges. This material is soluble in alcohol and water, and small quantities are carried over with the ethers and pass through the first filter into the beaker along with the ether-fat solutions. After the ethers have

been evaporated off and the beaker containing the extracted materials has been heated for one hour at 100°C. most of the non-fat material will be charred and stuck to the bottom. This dried, non-fat material is insoluble in anhydrous ether and petroleum benzin. The fat can then be completely separated from the non-fat material by redissolving it in ether and filtering it through a small filter paper.

It is difficult to keep ethyl ether anhydrous under practical conditions, and because of the possibility of redissolving some of the non-fat materials in the small quantities of water that it may contain, it was found to be advisable to use a mixture of equal parts of ethyl ether and petroleum benzin to redissolve the dried fat. Petroleum benzin (b.p. below 60°C.) has been found to be most satisfactory for use in washing the fat from the beaker and filter paper.

#### ACKNOWLEDGMENT

The cooperation of the following collaborators in this work is appreciated:

Gaston Dalby, Ward Baking Company, New York.

C. M. O'Malley, American Dry Milk Institute, Inc., Chicago, Ill.

R. H. Harter, E. K. Spotts, and E. G. White, National Biscuit Co. Laboratory, New York.

#### REPORT ON PROSTIGMINE

By F. J. McNALL (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

In accordance with the recommendations of sub-committee B, the work on prostigmine was continued with special reference to tablet mixtures of prostigmine bromide, and the use of the Volhard procedure for the determination of bromine. Collaborative results on the pure compound in good agreement were reported (*This Journal*, 25, 815).

Tablets of prostigmine bromide were secured from a reputable manufacturer and prepared by grinding in mortar and passing through a 60-mesh screen.

The method sent to collaborators is as follows:

#### METHOD

Weigh not less than 20 of the tablets, reduce them to a fine powder, and transfer an accurately weighed aliquot portion equivalent to ca. 0.15 gram of prostigmine bromide to a 300 ml. Erlenmeyer flask. Add 100 ml. of water, 5 ml. of HNO<sub>3</sub>, and 25 ml. of 0.05 N AgNO<sub>3</sub> solution. Add 2 ml. of ferric (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> indicator and titrate the excess AgNO<sub>3</sub> with 0.05 N KSCN. Each ml. of 0.05 AgNO<sub>3</sub> = 0.01516 gram of prostigmine bromide.

*Results of Collaborators*

COLLABORATOR	PROSTIGMINE BROMIDE
	per cent
1	6.48
	6.53
2	6.54
	6.48
	6.50
3	6.51
	6.53
4	6.59
	6.52
5	6.58
	6.61
Associate Referee	6.59
	6.57
Average	6.54

The Associate Referee wishes to express his appreciation to the following collaborators, all members of the U. S. Food and Drug Administration: (1) Jonas Carol, Chicago; (2) James B. Snider, Minneapolis; (3) Henry R. Bond, Kansas City; (4) Rupert Hyatt, Cincinnati; and (5) Iman Schurman, Cincinnati.

## DISCUSSION

The Volhard method as applied to tablets of prostigmine bromide gives excellent results as indicated by the close agreement among the collaborators. No adverse criticism was received from any of the analysts cooperating on this work.

## REPORT ON EMULSIONS

By HAROLD F. O'KEEFE (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

Work was planned in accordance with the recommendation made at the 1941 meeting, that the subject be further studied and that attention be given to the determination of the phenolphthalein in emulsion of liquid petrolatum with phenolphthalein by the iodination procedure. Since much difficulty was incurred by the Associate Referee and his collaborators in filtering the ether-alcohol solvent mixture, considerable work was also done on methods for the direct extraction of the phenolphthalein and the mineral oil from the emulsion without precipitating the gums. Several solvent mixtures were tried, but because of the large quantity of sample required for the phenolphthalein determination, troublesome emulsions were formed. Collaborator Orchin suggested in last year's report (*This*

*Journal*, 25, 843) that after the addition of the ether-alcohol solvent for precipitating the gums, the mixture be centrifuged until clear. The clear solvent mixture is then decanted directly into a separator without being filtered. This change in procedure was studied, and since it was found to effect a considerable saving of time and to produce good results, it has been incorporated into the method as submitted this year.

The official product contains vanillin and saccharin, and it was thought advisable to determine what effect, if any, their presence would have upon the results obtained by the iodination of phenolphthalein. Accordingly a control sample was prepared to contain these products in the same concentrations in which they appear in the official product, but omitting the phenolphthalein. Assay of this control by the same procedure used for the collaborative sample yielded no weighable residue. Collaborator Glycart suggested that the filtering of the ice-cold mixture through a Gooch in the iodination procedure be omitted. This suggestion has been incorporated into the method.

The materials for the work and the method of preparing the collaborative sample were the same as those given in last year's report (*This Journal*, 25, 843). The amount of phenolphthalein was calculated to be 0.472 per cent by weight.

Directions for the method and samples were submitted to the collaborators. The method follows:

#### METHOD

##### REAGENTS

*Solvent mixture*.—Mix 25 ml. of alcohol with 75 ml. of ether.

##### DETERMINATION

*Phenolphthalein*.—Shake the sample well (preferably in a mechanical shaker, for 10 minutes). Weigh accurately from a weighing buret, a quantity of sample equivalent to ca. 0.1 gram of phenolphthalein directly into a centrifuge bottle. Add 100 ml. of the solvent mixture, stopper the bottle, shake vigorously, and then centrifuge until clear. Decant into separator. Wash the residue in the bottle twice with 10 ml. portions of the solvent mixture, adding these washings to the separator. Dissolve the residue in the bottle in a few ml. of water and reprecipitate the gums with 50 ml. of the solvent mixture. Again shake and centrifuge as before, decanting into the separator. Wash the residue and bottle with three 10 ml. portions of the solvent mixture and add these to the separator. Dissolve the residue in a few ml. of water and test for complete extraction with NaOH.

Shake the solvent mixture in the separator repeatedly with ca. 0.1 *N* NaOH until the phenolphthalein has been completely removed as shown by the absence of color. Combine the alkaline extracts in a separator and acidify the solution by adding dilute H<sub>2</sub>SO<sub>4</sub>.

Extract the phenolphthalein by shaking the acid mixture repeatedly with 10 ml. portions of ether. Test for complete extraction with NaOH. Combine the ether extracts in a 150 ml. beaker, evaporate to dryness, and determine the phenolphthalein as directed in *Methods of Analysis*, A.O.A.C., 1940, 169, p. 613, omitting the operation, "filter the ice-cold mixture through a Gooch . . . washing several times with water."

*Collaborative Results*

COLLABORATORS*	PHENOLPHTHALEIN		AV.
	1 per cent	2 per cent	
J. H. Cannon, St. Louis	0.439	0.443	0.441
Jonas Carol, Chicago	0.478	0.470	0.474
C. K. Glycart, Chicago	0.474	0.477	0.476
George Keppel, Minneapolis	0.487	0.441	0.464
George McClellan, New Orleans	0.449	0.450	0.450
H. F. O'Keefe, Chicago	0.470	0.470	0.470
Sam Perlmutter, Minneapolis	0.475	0.455	0.465

\* All members of the U. S. Food and Drug Administration.

## COMMENTS OF COLLABORATORS

*C. K. Glycart.*—The method is rather long, but it is essential for accuracy. In the iodination of phenolphthalein, the directions "filter the ice-cold mixture in a Gooch . . . several times with  $H_2O$ " are not necessary, and it is suggested that the directions be amended to omit the step.

*George Keppel and Sam Perlmutter.*—Since no fat was present, the solution was not filtered just prior to final precipitation.

## DISCUSSION

Two methods for the determination of phenolphthalein in emulsion of liquid petrolatum with phenolphthalein have now been studied by the Associate Referee, and both have been submitted to collaborators. The quantitative results obtained have been approximately the same in both cases. The iodination procedure, however, eliminates the solubility factor involved in the first method when the phenolphthalein is precipitated in weak acid solution, filtered, and weighed as the pure compound. The time required for the assay is much shorter by the iodination method. Because an emulsion of this type is a difficult mixture to prepare and handle, the Associate Referee considers that the results of collaborative study have shown the iodination method to be a satisfactory one for use in assaying this product.

REPORT ON HALOGENS IN HALOGENATED  
FLUORESCEINS

By J. H. JONES (Cosmetic Division, U. S. Food and Drug  
Administration, Washington, D. C. ), *Associate Referee*

Last year a wet oxidation method was described<sup>1</sup> for the determination of chlorine and bromine in halogenated fluoresceins. A further study of this method, applied to the analysis of such compounds, has now been made.

<sup>1</sup> *This Journal*, 25, 944 (1942).

Experiments show that a 1 per cent solution of hydrazine sulfate is a more convenient absorption reagent in this procedure than is the sodium hydroxide-sodium sulfite solution previously recommended. With hydrazine sulfate the absorption of the evolved halogens is as complete as it is with sulfite, and there is no danger of contamination of the silver halide precipitate with silver sulfite. Also the determination of bromide may be carried out by Lang's method. This makes it possible to determine both bromine and chlorine in the sample.<sup>2</sup>

Some samples of hydrazine sulfate contain appreciable quantities of chloride, but a single recrystallization usually reduces the chloride content to a negligible amount. C. P. grades of the other reagents used ordinarily do not give an appreciable blank.

If a sufficiently accurate balance is available, the use of semimicro samples (25–50 mg.) is advantageous. With samples of this size, the oxidation never becomes violent; hence, the constant attention of the analyst is not required during the oxidation period. The procedure and apparatus described for the macro method may be used for semimicro samples without modification. It is, however, advisable to use a sintered-glass crucible for collecting the silver halide precipitate.

One advantage of the proposed method for chlorine and bromine is that these elements are separated from iodine at the time the sample is oxidized. However, iodinated fluoresceins tend to lose iodine readily when heated with sulfuric acid, and some iodine may be volatilized unless certain precautions are observed. In experiments where FD&C Red No. 3 (erythrosine) was used as the sample, it was found that this volatilization could be prevented if 25 ml. of sulfuric acid was used for each 50–75 mg. of sample. Apparently, under these conditions any iodine liberated remains dissolved in the sulfuric acid until oxidized to iodic acid. For compounds containing iodine it is recommended that semimicro samples be used. If this is not convenient the sample taken should be approximately 100 mg. and the amount of sulfuric acid increased to 50 ml. The use of this amount of acid does not decrease the accuracy of the method; the only disadvantage is that longer time (ca. 1 hour) is required for complete liberation of chlorine and bromine.

The proposed method appears to be convenient and accurate for compounds soluble in, but not volatilized from, hot concentrated sulfuric acid. Analysis of a number of compounds of this type, both macro and semimicro samples, indicates that an accuracy of 99.5 per cent is easily attained with a deviation between duplicates of  $\pm 0.5$  per cent.

A collaborative study of the rapid iodine method of Clark and Jones<sup>3</sup> has been undertaken.

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<sup>2</sup> See p. 433.

<sup>3</sup> *This Journal*, 25, 755 (1942).

Samples of FD&C Red No. 3, D&C Orange No. 16, and D&C Red No. 3 Aluminum Lake were sent with directions for analysis to the following collaborators:

Bates Chemical Co., E. Sheppard reporting.  
 H. Kohnstamm & Co., L. Koch and J. J. Morris reporting.  
 National Aniline and Chemical Co., J. D. Nantz reporting.  
 W. J. Stange and Co., W. H. Kretlow reporting.  
 U. S. Food and Drug Administration, F. L. Rotondaro, Drug Division,  
 G. R. Clark and L. A. Huard, Cosmetic Division, reporting.

Seven reports containing analytical results have been received to date. In addition, one analyst reported that his results with potassium iodide were too variable to warrant analyzing the dye samples. Six analysts reported that they were well satisfied with the accuracy and rapidity of the method. The results of these analysts and that of the Associate Referee are in fair agreement.

All results, listed in the order they were received, are shown in Table 1.

TABLE 1.—*Collaborative results for iodine in halogenated fluoresceins*

COLLABORATOR	FD&C RED NO. 3		D&C ORANGE NO. 16	D&C RED NO. 3 ALUMINUM LAKE
	"AS IS"	"IN COLOR ACID"	IODINE	IODINE
	IODINE	IODINE		
	per cent	per cent	per cent	per cent
1	51.6	60.7	23.4	9.0
2	51.3	60.6	23.2	9.0
3	50.8	59.7	22.8	8.8
4	31.0-43.2	33.9-47.3	2.6-4.2	4.4-5.4
5	56.0	62.5	23.3	9.1
6	51.5	60.7	23.3	9.1
7	51.6	60.7	23.3	9.2
8	51.7	60.8	23.4	9.2

The sample of FD&C Red No. 3 submitted had been purified by recrystallization, and the iodine content calculated on the basis of the color acid should be close to 60.7 per cent, the theoretical value. Five out of eight results for iodine in FD&C Red No. 3 are within  $\pm 0.2$  per cent of this theoretical value, one result is 1.5 per cent low and one 2.5 per cent high.

The sample of D&C Orange No. 16 was a commercial product. This sample contains bromine as well as iodine. Seven of the reported results for the iodine content are between 22.8 and 23.4 per cent, with six of the seven between 23.2 and 23.4 per cent.

The sample of D&C Red No. 3, aluminum lake, was a commercial product prepared by precipitation of FD&C Red No. 3 on alumina with



aluminum chloride. In this case seven of the values reported for the iodine content lie between 8.8 and 9.2 per cent, with six of the seven in the range 9.0 to 9.2 per cent.

The variation in results for the latter two dyes may be partly due to non-uniformity of the samples.

A possible explanation of the low results of two of the collaborators is that they failed to reduce all or nearly all the manganese dioxide before filtration. Manganese dioxide apparently absorbs or occludes iodine, and correct results can not be obtained if much manganese dioxide remains at the time the solution is filtered. This point probably was not sufficiently emphasized in the directions for analysis.

### CONCLUSIONS

The method appears to give accurate and reproducible results if the proper technic is used. Apparently, however, the directions need revision and more emphasis should be given to important steps in the procedure.

### Note on Determination of Iodine in Organic Compounds\*

Recently Weiner, Leach, and Bratz<sup>1</sup> published a method for the "Determination of Iodine in Tetraiodophenolphthalein," in which they refer to a procedure published by the writers in the August, 1942, issue of *This Journal* (p. 775) and give the impression that the procedure in the case of acid-insoluble alkali-soluble compounds employs a time-consuming oxidation by permanganate in alkaline solution.

To correct any misunderstanding an excerpt from page 758, Vol. 25, of *This Journal* is quoted:

*Nonvolatile compounds insoluble in acids but soluble in bases.*—Dissolve the sample in ca. 2 ml. of 30% NaOH solution, dilute to 100 ml., and add a few glass beads and 15 ml. of the saturated  $\text{KMnO}_4$ . Boil for 5 minutes and remove from heat. When boiling ceases, *add carefully 10 ml. of concentrated  $\text{HNO}_3$  and boil for 5 minutes more.* (The italics are the writers.)

The basis for this procedure, therefore, is an oxidation in acid solution and not in basic solution.

Weiner et al. also state that for *o*-iodobenzoic acid "it was necessary to replace the 45 minute digestion in alkaline permanganate by 8 hours of refluxing over a free flame." The writers agree fully that the oxidation of *o*-iodobenzoic acid proceeds very slowly in alkaline solution. Table 1, however, gives pertinent data about the application of the writers' procedure to *o*-iodobenzoic acid.

The time required for the complete analysis of each set of triplicate determinations was approximately one hour.

\* By G. R. Clark and J. H. Jones.

<sup>1</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 373 (1943).

TABLE 1.—*Iodine in o-iodobenzoic acid*

SAMPLE SIZE	THIOSULFATE TITRATION	NORMALITY	IODINE	
			FOUND	CALCULATED
<i>gram</i>	<i>ml.</i>		<i>per cent</i>	<i>per cent</i>
0.1541	36.40	0.1027	51.30	51.18
0.0794	18.70	0.1027	51.15	
0.0956	22.50	0.1027	51.12	
0.0873	40.20	0.0525	51.13	
0.0671	31.00	0.0525	51.30	
0.0652	30.05	0.0525	51.18	

Incidentally, in numerous checks on the oxidation of organic materials by this procedure the "blank" never exceeded 0.05 ml. of 0.05 *N* thio-sulfate and was usually nil. Hence the accuracy is not due to a balancing of errors.

## REPORT ON MASCARAS, EYEBROW PENCILS AND EYE SHADOWS

By JAMES W. FULLER (State Board of Barbers and Hairdressers,  
Augusta, Me.), *Associate Referee*

In accordance with the advice of the General Referee on Cosmetics, a preliminary report on mascaras, eyebrow pencils, and eye shadows is submitted, although only a beginning has been made on this extensive subject, and what has been done cannot be substantiated owing to the pressure of routine work of the collaborators.

### DEFINITIONS

(a) *Mascara*.—An eye cosmetic, either solid or liquid, usually black, brown, blue or green, used to color temporarily the eyelashes and occasionally the eyebrows.

(b) *Eyebrow Pencil*.—An eye cosmetic of the wax pencil type, usually black or brown, used to color temporarily the eyebrows.

(c) *Eye Shadow*.—An eye cosmetic of the cream type of varying shades of black, brown, blue, and green, used to color temporarily the eye-lids and surrounding area.

The following ingredients are used in making many of the mascaras, eyebrow pencils, and eye shadows:

#### (a) *Mascaras*:

##### *Solid Types*:

*Oils*: Liquid petrolatum, castor oil, olive oil.

*Waxes*: Ceresine, carnauba, ozokerite, and paraffin.

*Other Substances*: Alcohol, boric acid, glycerol, gum arabic, lanolin, oleic acid, petrolatum, rice starch, ricinoleic acid, stearic acid, soap, and water.

##### *Liquid Types*:

Alcohol, balsam Peru, castor oil, liquid petrolatum, tincture benzoin, weak aqueous shellac.

The dyes and pigments used in both of the above types include among the browns burnt sienna, burnt umber, ferric oxide, Indian red, terra umber, coal-tar color lakes, and ferric oxide; among the blacks, lampblack, bone black, drop black, vine black, and charcoal black; among the blues, ultramarine blue and titanium oxide; and among the greens, usually chrome green, occasionally a green coal tar dye. See below "Detection of Coal-Tar Dyes."

(b) *Eye-brow Pencils:*

Benzoated tallow, cocoa butter, Japan wax, olive oil, petrolatum, hard and soft paraffin, petroleum oil, and white wax.

*Pigments:*

Barium sulfate, lampblack, ocher, titanium oxide, umber, and zinc oxide.

(c) *Eye Shadows:*

Beeswax, cetyl alcohol, lanolin, paraffin, petrolatum, liquid petrolatum, and spermaceti.

*Pigments:*

Aluminium powder, artificial indigo, brilliant green lake, carbon black, China clay, chrome green, chrome blue G. C. B., methyl violet lake, Prussian blue, red iron oxide, ultramarine blue, ultramarine violet, yellow iron oxide, and other coal tar dyes.

The following mascaras, eyebrow pencils, and eye shadows were made, because a sufficient amount for testing was unobtainable from other sources, except in isolated cases where cosmetic companies had submitted a sample of sufficient size.

I. *Mascara Brown Type 1*

	grams
Stearic acid powdered U.S.P.	140
Carnauba wax	106
Ozokerite	84
Petrolatum white	42
Castor oil	42
Olive oil	42
Triethanolamine	84
Liquid petrolatum	210
Beeswax	50
Burnt umber	80
Burnt sienna	7
Indian red	21

II. *Mascara Base Type 2*

Stearic acid	30
Carnauba wax	42.8
Ozokerite	10.8
Petrolatum	40
Petrolatum, liquid	40
Triethanolamine	30
Beeswax	2.8
Japan wax	40
Cocoa butter	24

	<i>grams</i>
Oleic acid	10
a. Brown	
Burnt Umber	20
b. Blue	
Blue Prussian Blue	20
BaSO <sub>4</sub>	10

### III. Eyebrown Pencil Base

Liquid petrolatum	60
Cocoa butter	36
Japan wax	60
Petrolatum	60
Ceresine	15
Beeswax	4
Carnauba wax	64

a. Black. To 300 grams of formula 10 grams of lampblack was added.

b. Brown. To 300 grams of formula 15 grams of burnt umber was added.

### IV. Eye Shadow Base

	<i>grams</i>
Liquid petrolatum	250
Lanolin	100
Beeswax	50
Paraffin	100

a. *Brown No. 1.*—To 150 grams of base 20 grams of an iron ochre pigment was added.

b. *Brown No. 2.*—To 150 grams of base were added 5 grams of Brown No. 78 and 10 grams of BaSO<sub>4</sub>.

c. *Gray.*—To 150 grams base of gray were added 30 grams of TiO<sub>2</sub>, 30 grams of BaSO<sub>4</sub>, 5 grams of lampblack, and 40 grams of talc.

d. *Green.*—To 150 grams of green were added 10 grams of green dye and 10 grams of TiO<sub>2</sub>.

e. *Blue.*—To 150 grams of blue were added 10 grams of ultramarine blue, 5 grams of TiO<sub>2</sub>, and 5 grams of BaSO<sub>4</sub>.

### V. Blank.

A mixture of the base ingredients in the greatest proportion used in any formula.

	<i>grams</i>
Stearic acid	35
Carnauba wax	26
Ceresine	21
Petrolatum	15
Castor oil	10
Olive oil	10
Triethanolamine	21
Liquid petrolatum	62
Beeswax	12
Paraffin	25
Japan wax	15
Cocoa butter	9
Lanolin	25

A group of samples was selected, some submitted by cosmetic companies and others made according to the above formulas. In making the samples the Associate Referee found it to be extremely difficult with the apparatus available to get an even distribution of the solid inorganic pigments throughout the wax and oil bases. To some extent this might account for variation of the results in a few of the duplicate samples.

*List of Samples*

1. Liquid Winx Brown					
2. Liquid Winx Black					
3. Eye Shadow Blue					
4. Cream Mascara Brown					
5. Cream Mascara Black					
6. Cake Winx (Mascara) Brown					
7. Barbara Gould Brown Eye Shadow					
8. Barbara Gould Mascara					
9. Eyebrow Pencil Brown—Made according to formula IIa					
10. Eyebrow Pencil Black	"	"	"	"	IIb
11. Eye Shadow Blue	"	"	"	"	IIIe
12. Eye Shadow Green	"	"	"	"	IIId
13. Eye Shadow Brown	"	"	"	"	IIIa
14. " " "	"	"	"	"	IIIb
15. Eye Shadow Gray	"	"	"	"	IIIf
16. Mascara Brown	"	"	"	"	Ib
17. " "	"	"	"	"	Ia
18. " "	"	"	"	"	Ic
19. Blank	"	"	"	"	IV

Some of the harmful and deleterious substances likely to be found in eyebrow pencils, eye shadows, and mascara, outside of monomethyl ether, are arsenic, soluble barium and chromium compounds, coal tar dyes, and in exceptional cases compounds of silver and heavy metals.

### I. GENERAL QUALITATIVE PROCEDURE

Since the base materials are in all cases very similar, the procedure to be used in the examination of mascaras, eyebrow pencils, and eye shadows should be generally applicable to all three types of cosmetics.

a. *Physical Examination of Sample.*—The sample should be described as to weight, color, odor, and general physical appearance.

b. *Qualitative Test for Metals.*—A careful spectroscopic examination of the ash from approximately one gram of the sample should give good results for all metals likely to be encountered, except sulfur and arsenic. The metallic elements present give an indication of the mineral pigments or color bases that may be present.

c. *Detection of Coal Tar Dyes.*—The Coal Tar Color Regulations,<sup>1</sup> issued September 1940, under authority of the Federal Food, Drug, and Cosmetic Act of 1938, state that no product containing coal tar dyes shall be used in the area of the eye. It therefore becomes purely a question of testing mascaras, eyebrow pencils, and eye shadows for the presence of coal tar dyes. The procedure follows:

<sup>1</sup> S.R.A.F.D.C. No. 3. U. S. Food and Drug Administration.

Treat a small sample with water, heat to boiling, and filter to a clear solution. If the filtrate is colored, divide into equal portions. Make the first portion slightly acid with HCl (1+9) and introduce a reasonably large piece of wool yarn. Heat to boiling, boil for ca. 5 minutes, remove the wool, and wash well. If the wool is dyed place in 5 ml. of  $\text{NH}_4\text{OH}$  (1+50) and heat to boiling.

Remove the wool, and if the ammonia is colored make it slightly acid with the HCl and introduce another small piece of wool. If this piece is found to be colored on boiling and washing, a water-soluble, acid, coal-tar dye is indicated. Make the second portion slightly ammoniacal with the  $\text{NH}_4\text{OH}$  and treat exactly the reverse of the above process. (If the wool is dyed, a water-soluble, basic, coal-tar dye is indicated.)

NOTES: In a few cases the dye or pigment may be found in a dilute shellac, gum benzoin, resin, or gum and soap mixture. If so a pigment may be held in a fine suspension when treated with water and in a filtration may be carried through the filter. If the filtrate of such a mixture is acidified and wool is added, the pigment or dye collects on the wool, giving it the appearance of being dyed. The wool in such cases should be removed and boiled with ca. 10 ml. of alcohol. If it still appears to be dyed, apply the wool-dyeing test as directed previously.

One exception to the above case is an oil- or spirit-soluble dye. If the dye is insoluble in water, treat the residue after filtration of the water solution with 10 ml. of 95% alcohol, heat to boiling, and filter. If the filtrate is colored, slightly acidify with the HCl and perform the dyeing and stripping test as directed for the water solution. Some lakes will give slightly colored solutions in both water and alcohol, but these solutions will not respond to the dye test. The alcohol solution may be highly colored and yet not dye well, in which case it will be necessary to treat a new sample by the following method for lakes:

Treat a small sample with ca. 20 ml. of a 20% solution of  $\text{Na}_2\text{CO}_3$ , heat to boiling for 1 minute, filter, and acidify the filtrate with acetic acid (1+10), making just acid. If the filtrate is colored, perform the wool dyeing and stripping test. If the filtrate is not colored, treat a new sample with the dilute HCl, boil for a few minutes, and filter. If a colored filtrate is obtained, make the solution to nearly neutral with the dilute  $\text{NH}_4\text{OH}$ , and perform the wool dyeing and stripping test. If all the above tests are negative, indicating a mineral pigment, its nature may be investigated by inorganic qualitative analysis.

## II. QUANTITATIVE DETERMINATION OF VOLATILE MATTER, SOLID MATTER, ASH, ARSENIC AND TITANIUM OXIDE

a. *Volatile Matter, Solid Matter, and Ash.*—Weigh a sample of ca. 1 gram into a platinum dish, heat for 5 hours in an oven at  $100^\circ\text{C}$ ., cool, and reweigh. (Heating at this temperature was necessary to bring the residues to nearly constant weight. Even then the samples continued to lose weight slowly owing to the volatilization of the fats and oils. However, heating for 5 hours at  $100^\circ\text{C}$ . seemed to give a fairly accurate indication of the solid matter present.) Carefully ignite the residue from the determination of volatile matter to a white ash at low heat, weigh the remaining material, and calculate to percentage of ash.

The results of the determination of the solid matter, volatile matter by difference, and ash are shown in Table 1.

b. *Arsenic.*—The official Gutzeit method (*Methods of Analysis*, A.O.A.C., 1940, p. 390) was followed exactly. However, from experiments conducted on arsenic in hair tonics by Harold Burrill of this laboratory it would appear that much work still remains to be done on the determination of arsenic in samples rich in organic material, such as fats and waxes. In the preparation of the sample for the Gutzeit test

TABLE 1.—Results on solid matter, volatile matter, and ash

SAMPLE NO.	WT. OF SAMPLE	SOLIDS		VOLATILE MATTER		ASH		
		grams	per cent	grams	per cent	ml.	per cent	as. per cent
1—a	5.4348	1.1405	20.98	79.02	79.02	0.2197	4.04	
1—b	4.0589	0.8446	20.81	20.89	79.19	0.1648	4.06	4.05
2—a	1.9784	0.3479	11.98		88.02	0.0446	2.25	
2—b	1.2638	0.2214	11.94	11.96	88.06	0.0288	2.28	2.26
3—a	0.6901	0.6744	97.73		2.27	0.1276	18.47	
3—b	0.8149	0.7959	97.68	97.70	2.32	0.1506	18.46	18.45
4—a	1.0646	0.9946	93.40		6.60	0.0309	2.91	
4—b	1.1633	1.0809	92.95	93.17	7.05	0.0572	3.35	3.13
5—a	1.0534	0.9411	89.44		10.56	0.0007	0.665	
5—b	1.0579	0.9517	90.71	90.07	9.29	0.0008	0.750	0.707
6—a	0.6058	0.5965	98.46		1.54	0.2064	34.07	
6—b	0.6025	0.5932	98.47	98.46	1.53	0.2059	34.16	34.11
7—a	1.1060	1.1000	99.48		0.52	0.1423	12.92	
7—b	1.3075	1.2986	99.30	99.39	0.60	0.1675	12.88	12.90
8—a	0.9277	0.9116	98.25		1.75	0.1425	15.36	
8—b	1.2092	1.1895	98.36	98.30	1.64	0.1893	15.66	15.51
9—a	1.8744	1.8465	98.48		1.52	0.1822	9.73	
9—b	1.2383	1.2242	98.86	98.67	1.14	0.1188	9.61	9.67
10—a	1.0822	1.0719	99.02		0.98	0.0002	0.018	
10—b	1.5446	1.5271	98.90	98.96	1.10	0.0004	0.026	0.022
11—a	1.0325	1.0195	98.76		1.24	0.1050	10.17	
11—b	1.5455	1.5279	98.90	98.83	1.10	0.1527	9.89	10.03
12—a	1.3349	1.3103	98.21		1.79	0.1313	9.83	
12—b	1.8813	1.8635	98.20	98.20	1.80	0.1868	9.94	9.88
13—a	1.5303	1.5125	98.72		1.28	0.1013	6.62	
13—b	1.2844	1.2685	98.70	98.71	1.20	0.0940	6.63	6.625
14—a	1.5146	1.4809	97.78		2.22	0.0981	6.50	
14—b	1.3862	1.3505	97.44	97.61	2.56	0.0943	6.80	6.65
15—a	2.3516	2.2995	97.80		2.20	0.7818	33.27	
15—b	2.0464	1.9905	97.30	97.50	2.70	0.6868	33.57	33.42

by destruction of organic matter with concentrated sulfuric and nitric acids there appears to be a considerable loss of arsenic.

The results of the arsenic determinations are shown in Table 2. The samples were run in duplicate, and it was considered necessary to take small samples owing to the difficulty in destroying the organic matter.

c. *Titanium Oxide*.—Titanium oxide is used as a pigment or a lake base in eye shadows, eyebrow pencils, and mascaras. The quantity present can be determined with ease and reasonable accuracy and should be determined even though the harmfulness of the oxide is negligible.

The results of the determinations of titanium in Samples 11, 12, and 15 made by the peroxide colorimetric method are shown in Table 3. The method follows:

#### TITANIUM

##### REAGENTS

*Standard titanium oxide solution*.—Weigh 0.5 gram of  $\text{TiO}_2$  and fuse with 10–15 grams of  $\text{KHSO}_4$  in a platinum dish, keeping the mixture at fusion temperature

TABLE 2.—*Results on arsenic*

SAMPLE NO.	WT. OF SAMPLE	MADE TO—	ALIQOUT TAKEN	EQUIVALENT $\text{As}_2\text{O}_3$		
				mg.	per cent	av. per cent
1—a	0.9019	100	5 & 10	0.003 —0.006	0.00665	
1—b	1.3292	100	5 & 10	0.004 —0.008	0.00604	0.0064
2—a	1.1265	100	5 & 10	0.0025—0.0045	0.00418	
2—b	1.0889	100	5 & 10	0.002 —0.004	0.00367	0.0039
3—a	0.3640	direct		0	0	
3—b	0.7110	direct		0	0	0
4—a	0.9565	100	50 & 5	too heavy—0.003	0.0063	
4—b	1.0299	100	50 & 10	too heavy—0.007	0.0068	0.0065
5—a	0.8063	direct		0	0	
5—b	1.0259	direct		0	0	0
6—a	0.5250	100	5 & 10	0.003 —0.008	0.0134	
6—b	0.4756	100	5 & 10	0.003 —0.009	0.0157	0.0145
7—a	1.6732	direct		0	0	
7—b	1.2424	direct		0	0	0
8—a	0.9751	direct		0.001	0.00012	
8—b	0.8233	direct		0.0005	0.00006	0.00009
9—a	0.6816	100	50 & 10	too heavy—0.012	0.00176	
9—b	0.8597	100	50 & 10	too heavy—0.015	0.00174	0.00175
10—a	0.9654	direct		0	0	
10—b	1.0442	direct		0	0	0
11—a	0.7681	direct		0	0	
11—b	0.9486	direct		0	0	0
12—a	1.3490	direct		0	0	
12—b	1.1911	direct		0	0	0
13—a	1.1746	direct		0	0	
13—b	1.1644	direct		0	0	0
14—a	1.1094	direct		0	0	
14—b	1.2609	direct		0	0	0
15—a	1.0055	direct		0	0	
15—b	1.3923	direct		0	0	0
16—a	0.9047	100	5 & 10	0.002 —0.003	0.0044—0.0033	
16—b	1.0665	100	5 & 10	0.0015—0.0025	0.0028—0.0023	0.0032
17—a	1.1977	100	5 & 10	0.015—too heavy	0.025	
17—b	1.1157	100	5 & 10	0.011—0.02	0.0188	0.022
18—a	1.8729	direct		0	0	
18—b	1.2702	direct		0	0	0
19—a	2.1891	direct		0	0	
19—b	1.3854	direct		0	0	0

until all the oxide is dissolved. Dissolve the fusion in  $\text{H}_2\text{SO}_4$  (1+19), heating gently if necessary; transfer to a 500 ml. volumetric flask; cool, and make to 500 ml. with the  $\text{H}_2\text{SO}_4$ . (1 ml. of this solution contains 0.001 gram of  $\text{TiO}_2$  or 0.0006 gram of  $\text{Ti/ml.}$ ).

*Hydrogen peroxide.*—Regular 3% solution of  $\text{H}_2\text{O}_2$  made by the  $\text{BaO}_2$  method.

#### PROCEDURE

Weigh ca. 0.5 gram of sample directly into a platinum dish, ash over a low flame,



add 10 grams of  $\text{KHSO}_4$ , and fuse carefully at low heat to prevent spattering. Allow the sample to remain at fusion temperature for ca. 15 minutes.

Cool, take up the fusion with 20 ml. of  $\text{H}_2\text{SO}_4(1+19)$ , heat to boiling, and filter into a 50 ml. Nessler tube. Wash the platinum dish with a 20 ml. portion of the  $\text{H}_2\text{SO}_4$ . Add to a Nessler tube 5 ml. of 3%  $\text{H}_2\text{O}_2$ , make to mark with the  $\text{H}_2\text{SO}_4$ , and compare with standards.

Prepare standards with 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 ml. of the standard  $\text{TiO}_2$  solution, add 5 ml. of the 3% peroxide, and make to 50 ml. with the  $\text{H}_2\text{SO}_4$ . If necessary, make additional standards in the same manner or dilute the samples with the  $\text{H}_2\text{SO}_4$  as standards high in  $\text{TiO}_2$  cannot be read with accuracy.

TABLE 3.—Results on Samples 11, 12, and 15 by peroxide colorimetric method

SAMPLE NO.	WT. OF SAMPLE	EQUIVALENT $\text{TiO}_2$		
		mg.	per cent	av. per cent
11	1	0.5400	8.	1.48
	2	0.4823	7.2	1.49
	3	0.2652	4.	1.51
12	1	0.2803	5.2	1.85
	2	0.5361	10.	1.86
	3	0.2937	5.5	1.87
15	1	0.6630	45.5	6.87
	2	0.3123	21.5	6.88
	3	0.7243	50.	6.92

For any elements likely to be found in eye shadows, eyebrow pencils, and mascaras that will interfere with the above procedure, such as chromium and iron, fuse the sample with  $\text{Na}_2\text{CO}_3$ , leach the fusion with water, filter, fuse the residue with  $\text{KHSO}_4$ , and treat by the regular procedure.

## REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

### POTASSIUM

During the past several years Associate Referees Gerritz<sup>1</sup> and Wood<sup>2</sup> of this Association have clarified, simplified, and subjected to collaborative study several procedures for the determination of potassium in fruits and fruit products. The present A.O.A.C. procedures were developed from Tilden's<sup>3</sup> modification of the Lindo-Gladding (potassium chloroplatinate) method or from the Wilcox<sup>4</sup> (cobaltinitrite) procedure. The analyst

<sup>1</sup> *This Journal*, 25, 232, 433 (1942).

<sup>2</sup> *Ibid.*, 429; 24, 391, 455 (1941); see also p. 472.

<sup>3</sup> *Ibid.*, 12, 366 (1929); 6, 399 (1923).

<sup>4</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 136 (1937).

now has a choice of two methods of sample preparation and several procedures for the determination of potassium.

The sample may be prepared by ashing in a platinum or nickel dish at 525°–550°C., either with or without the use of magnesium nitrate as an ash aid. After solution of the ash in dilute acid (HCl or HNO<sub>3</sub>) potassium may be determined either by the official gravimetric chloroplatinate or the cobaltinitrite procedure, or by shortened gravimetric or volumetric procedures that omit the time-consuming operation of removing calcium and magnesium from the ash solution before precipitation of the potassium. The methods of sample preparation and potassium determination that have been found suitable are assembled in this report.

St. John and Midgley<sup>5</sup> suggest wet digestion of the samples in a beaker with nitric followed by perchloric acid. This method of sample preparation was followed exactly as described and compared with the regular A.O.A.C. ashing procedure (525°C. in a platinum dish) by Ramsey, Assistant Chemist, U. S. Food and Drug Administration. The potassium in the sample, a currant jelly juice, was determined by the A.O.A.C. gravimetric chloroplatinate procedure. Wet digestion in a beaker gave on calculation 207 and 214 mg. of potassium oxide per 100 gram sample, while the official procedure gave 247 and 249 mg. of potassium oxide for duplicate determinations. The Referee made a wet digestion of the same sample, using nitric acid with 0.5 ml. of sulfuric acid followed by perchloric acid (otherwise as directed by St. John and Midgley<sup>5</sup> for sample preparation). The potassium in the digest was determined by the A.O.A.C. cobaltinitrite method. This procedure gave 183 and 196 mg. of potassium oxide per 100 grams of sample. These preliminary results indicate losses, by wet digestion in glass, of 15 and 23 per cent. Gerritz<sup>1</sup> found that wet digestion of fruit samples with nitric and sulfuric acids is successful if the final removal of acid is made in a platinum dish, but that removal of the acid by heating in Pyrex, porcelain, or silica leads to erratic and usually low potash results.

In the precipitation of potassium as chloroplatinate, neither Gerritz<sup>1</sup> nor St. John and Midgley<sup>5</sup> removed calcium and magnesium prior to precipitation. Wood obtained collaborative data by this modification for comparison with potassium results on the same samples analyzed according to the regular A.O.A.C. gravimetric chloroplatinate and cobaltinitrite procedures. The data are summarized in Table 1. The values for potash by all three procedures are in excellent agreement. Additional collaborative data obtained by analysts of the U. S. Food and Drug Administration are shown in Table 2. It is evident that the shorter gravimetric chloroplatinate modification is suitable for the determination of potash in fruits and fruit products.

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<sup>5</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 301 (1942).

The suitability and interchangeability of the several methods of potash determination are also illustrated by the results of analysis of three fruit samples shown in Table 3. These data and the reports of the Associate Referees on Potassium in Fruits and Fruit Products show that it is now

TABLE 1.—*Collaborative data on potash obtained on three samples by three methods*  
(mg. K<sub>2</sub>O/100 grams)

METHOD AND COLLABORATOR	SAMPLE A RASPBERRY JUICE			SAMPLE B GRAPE JUICE			SAMPLE C JELLY		
	OFFICIAL CHELORO- PLATINATE	SHORT- ENED CHELORO- PLATINATE	COBALTI- NITRITE	OFFICIAL CHELORO- PLATINATE	SHORT- ENED CHELORO- PLATINATE	COBALTI- NITRITE	OFFICIAL CHELORO- PLATINATE	SHORT- ENED CHELORO- PLATINATE	COBALTI- NITRITE
San Francisco	181	184	180	86	85	85	149	149	148
H. W. Gerritz	181	183	179	85	85	87	147	149	145
Washington			183*						
L. L. Ramsey			185*	82	83		151	151	153
			186	83	84	85	152	152	153
Philadelphia									152
H. Shuman	175	178	173	80	81	82	144	147	153
New York	187	180	180	87	84	85	148	148	145
C. A. Wood	187	182	180	84	85	85	151	149	145
Maximum	187	184	186	87	85	87	152	152	153
Average	182	181	181	84	84	85	149	149	149
Minimum	175	178	173	80	81	82	144	147	145

\* R. A. Osborn.

advisable to assemble the procedures of sample preparation and determination somewhat as follows:

#### POTASSIUM IN FRUITS AND FRUIT PRODUCTS

##### 1

##### PREPARATION OF SAMPLE

(a) *Total ash (official)*.—Ash 15–30 grams of sample (representing ca. 15 grams of fruit) as directed in *Methods of Analysis, A.O.A.C.*, 1940, 337, 9.

(b) *Rapid ash*.—To 15–30 grams of sample in a nickel or platinum dish (preferably flat-bottomed, 3½ inches in diameter, and 1 inch high), add 1 ml. of 25% Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O solution; evaporate, char, and heat at a temperature not exceeding 550°C. until carbon is removed (ca. 15 minutes). Cover dish with a watch-glass and cool.

TABLE 2.—*Potassium in fruits and fruit products—comparison of procedures*

SAMPLE	ANALYST	MG. K <sub>2</sub> O/100 GRAMS		
		REGULAR GRAV.	SHORT GRAV.	GRAV.
		CHLOROPLATINATE PROCEDURE	CHLOROPLATINATE PROCEDURE	COBALTINITRITE PROCEDURE
Black Raspberries	D. Banes	260.3	261.6	
Black Raspberries	R. Martens	256.3	262.3	
Raspberry Jelly	D. Banes	157.6	165.3	
Raspberry Jelly	R. Martens	162.5	166.5	
Pineapple Crushed	D. Banes	183.8	193.1	
Pineapple Crushed	R. Martens	188.6	192.6	
Pineapple Jam	D. Banes	94.7	96.1	
Pineapple Jam	R. Martens	93.5	95.1	
Red Raspberries	D. Banes	204.9	211.3	
Red Raspberries	D. Banes	204.7	210.7	
Pineapple	R. A. Dick	128.0	131.6	
Red Currants	S. D. Fine	263.0	264.0	273.0
Grapes	S. D. Fine	279.0	281.0	277.0
Grape Juice	S. D. Fine	147.0	149.0	153.0
Red Raspberry	S. D. Fine	268.0	266.0	269.0
Jelly Juice				
Red Raspberries	S. D. Fine	240.0	238.0	241.0

TABLE 3.—*Comparison of sample preparation and determination of K<sub>2</sub>O  
(mg. K<sub>2</sub>O/100 grams)*

PREPARATION DETERMINATION	OFFICIAL ASH REGULAR GRAV. CHLOROPLATINATE	RAPID ASH		
		GRAV.	INDIRECT VOL.	SHORT GRAV.
		COBALTINITRITE	CHLOROPLATINATE	CHLOROPLATINATE
Samples				
Blackberry	260	255	258	
	261	257	260	
Loganberry	151	149	148	151
	152	150	149	153
Apricot	425	429	424	429
	430	435	424	435

L. L. Ramsey, Analyst.

*Chloroplatinate Methods*

## 2

## REAGENTS

(a) *Formic acid*.—A. C. S. reagent grade.(b) *Chloroplatinic acid*.—Dissolve 4.4 grams of H<sub>2</sub>PtCl<sub>6</sub> (equivalent to 2.1 grams of Pt) in water and dilute to 100 ml. 1 ml. of this solution = 10 mg. of K<sub>2</sub>O. Use ca. 20% excess.

(c) *Ammonium chloride-potassium chloroplatinate solution*.—Dissolve 200 grams of  $\text{NH}_4\text{Cl}$  in water and dilute to 1 liter. Add 5–10 grams of pulverized  $\text{K}_2\text{PtCl}_6$ , shake mixture at intervals for 6–8 hours, allow to settle, and filter. (The undissolved  $\text{K}_2\text{PtCl}_6$  may be used again.)

(d) *Alcohol*.—90%. Dilute  $\text{C}_2\text{H}_5\text{OH}$  with water to sp. gr.  $20^\circ/20^\circ$  of 0.829–0.832. Use in a wash bottle.

(e) *Calcium carbonate suspension*.—Mix 50 ml. of  $\text{C}_2\text{H}_5\text{OH}$  with 50 ml. of glycerol and add 50 grams of  $\text{CaCO}_3$  powder. Keep in a dropping bottle and shake vigorously before using.

(f) *Calcium formate suspension*.—Mix 50 ml. of  $\text{C}_2\text{H}_5\text{OH}$  with 50 ml. of glycerol and add 50 grams of  $\text{Ca}(\text{CHO}_2)_2$  fine crystals. Keep in a dropping bottle and shake vigorously before using.

(g) *Alcoholic sodium hydroxide and sodium formate*.—Shake  $\text{NaOH}$  pellets with  $\text{C}_2\text{H}_5\text{OH}$  containing 0.2 ml. of formic acid per 100 ml. of alcohol until saturated.

(h) *Nitric acid*.—(1+1). Mix equal parts of  $\text{HNO}_3$  A.C.S. reagent grade and water, boil to remove oxides, and cool.

(i) *Ferric alum, saturated*.—Shake 100 grams of  $\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$  with ca. 100 ml. of hot water, cool, and filter.

(j) *Silver nitrate solution*.—0.1 *N*. Dissolve 16.989 grams of  $\text{AgNO}_3$  and dilute to 1 liter with recently boiled water. Standardize against pure  $\text{NaCl}$  by the A.O.A.C. method (*This Journal*, 24, 101).

(k) *Potassium thiocyanate solution*.—Dissolve 9.717 grams of  $\text{KCNS}$  in water, dilute to 1 liter, and standardize against  $\text{AgNO}_3$ .

### 3

#### DETERMINATION

Wet down the ash 1 (a) or (b) with 5–10 ml. of distilled water, cover dish with watch-glass, and acidify with a slight excess of 1+4  $\text{HCl}$  (2–3 ml. for 1 (a) and 4–5 ml. for 1 (b)). For volumetric chloroplatinate method proceed from this point as directed under 3(b); for the short gravimetric method proceed as directed in the paragraph designated by the \*.

(a) *Gravimetric chloroplatinate method*.—Rinse watch-glass into dish and evaporate ash to dryness on steam bath. Add 5 drops of  $\text{HCl}$  (1+1) to the residue. Add 5–10 ml. of hot water and rub sides and bottom of container with policeman (rubber-tipped rod). Transfer the ash solution to a 250 ml. beaker with 50–75 ml. of hot water. Add a few glass beads and heat to boiling. Make distinctly alkaline with  $\text{NH}_4\text{OH}$  and add sufficient saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  for complete precipitation (usually not more than 1 ml.), cover beaker, and heat until precipitate becomes sufficiently granular to filter readily (incipient boiling for 30 minutes usually sufficient). Filter through 5 or 7 cm. fine-textured filter paper into a large platinum dish and wash thoroughly with hot water (5–6 fillings of filter are usually sufficient).

Evaporate solution nearly to dryness on steam bath and add 1 ml. of  $\text{H}_2\text{SO}_4$  (1+1). So rotate dish that the  $\text{H}_2\text{SO}_4$  comes in contact with all residue, adding a little water if necessary. Return dish to steam bath and evaporate all the water possible at that temperature. Then heat the dish preferably on a hot plate at a temperature of ca.  $150^\circ$  until bubbling caused by decomposition of oxalates ceases, and gradually increase the temperature until the  $\text{H}_2\text{SO}_4$  has evaporated. (When properly controlled this treatment usually takes 45–90 minutes.) Heat the sample cautiously over a burner, being careful to avoid loss due to sputtering during the decomposition of the ammonia compounds. Finally heat the dish to redness to remove traces of  $\text{NH}_4$  and complete the ignition. Add 5 drops of  $\text{HCl}$  (1+1) to the residue.

\* Transfer the ash solution to a round-bottomed porcelain dish of 100–200 ml. capacity, using ca. 50 ml. of hot water. Add a small excess of  $\text{H}_2\text{PtCl}_6$  solution. Place mixture on steam bath and rotate dish from time to time to prevent precipitate from baking on the side of the dish, and evaporate to a pasty consistency. (It is advisable to start the evaporation with several of the steam bath rings removed, and as the concentration progresses to replace the rings so that the heat is applied only to that surface of the dish that is covered by liquid.) Avoid exposure to  $\text{NH}_3$  fumes at all times. Add ca. 50 ml. of 90% alcohol to the dish and transfer to a Gooch crucible containing a suitable asbestos mat or Gooch crucible with fritted disk—30 ml. capacity, medium porosity. Wash 8 or 10 times with 20 ml. portions of 90% alcohol, then 5 or 6 times with 10 ml. portions of the  $\text{NH}_4\text{Cl}-\text{K}_2\text{PtCl}_6$  solution. Again wash well 6 or 8 times with 20 ml. portions of 90% alcohol.† Dry ca. 30 minutes in a  $100^\circ\text{C}$ . oven, cool, and weigh. Wash the  $\text{K}_2\text{PtCl}_6$  through the Gooch with hot water, using slight suction; then wash the Gooch with alcohol, dry, cool, and weigh. Difference in weight  $\times 0.19376 = \text{K}_2\text{O}$ .

Report results as mg./100 grams of original sample.

(b) *Volumetric-chloroplatinate method.*—Transfer the ash solution to a round-bottomed porcelain dish of 100–200 ml. capacity, using hot water. If carbon or other insoluble material remains in the ash, filter into the dish through a 5.5 cm. medium-textured paper, washing the metal dish and filter 4 or 5 times with 5 ml. portions of hot water. Evaporate (rapidly if desired) to 10 or 15 ml., add the  $\text{H}_2\text{PtCl}_6$  solution in excess, and evaporate on a steam bath to a heavy consistency (impinge a stream of air on the surface of the liquid to hasten evaporation, and rotate the dish from time to time to wash the crystals into the center). Cool the dish, and if crystals become dry on cooling, add a drop of 1+4  $\text{HCl}$  and 2 drops of water, so that the mass remains moist and holds the salts in solution. Add ca. 10 ml. of 90% alcohol, triturate with a policeman, and decant immediately onto a prepared‡ Gooch crucible. Wash the dish and crystals once or twice more with ca. 5 ml. portions of 90% alcohol, and then transfer the precipitate to the Gooch. Wash crucible free of  $\text{H}_2\text{PtCl}_6$  with 90% alcohol and then wash three or four times with 5 ml. portions of the  $\text{NH}_4\text{Cl}$  solution, pouring the solution gently into the crucible from the graduate so that the  $\text{CaCO}_3$  mat is not disturbed. Wash the  $\text{NH}_4\text{Cl}$  from the crucible with 4 or 5 washings of 90% alcohol. Cover the  $\text{K}_2\text{PtCl}_6$  completely with the  $\text{Ca}(\text{CHO}_2)_2$  by adding ca. 1 ml. of the suspension. Remove the liquid with suction and wash once with alcohol. Cover with  $\text{Na}_2\text{CO}_3$  to a depth of 2–3 mm. and moisten with ca. 1 ml. of alcoholic  $\text{NaOH}$ . Ignite at ca.  $500^\circ\text{C}$ . for 5–10 minutes.§

Cool the Gooch, place in a filter attachment, add ca. 5 ml. of hot water, and

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\* Beginning of short gravimetric chloroplatinate method.

† *Titration of potassium chloroplatinate precipitate.*—Dissolve the precipitate in the Gooch with several portions of boiling distilled water. Stir gently to facilitate solution and using suction collect filtrate and washings in a 250 ml. wide-mouthed, lipped Erlenmeyer flask. Add 1 ml. of the formic acid, heat to boiling, and simmer for ca. 2 minutes after the metallic Pt has formed. Add 10 ml. of (1+1)  $\text{HNO}_3$  mix, and then add a small excess of standard  $\text{AgNO}_3$  accurately measured. Boil vigorously for 5 minutes, cool, and filter through a Gooch crucible with fritted disk of medium porosity. Wash precipitate 5 or 6 times with 2%  $\text{HNO}_3$  solution, breaking up lumps with a glass rod, and collect filtrate and washings in an Erlenmeyer flask. Add 5 ml. of saturated ferric alum indicator and with vigorous agitation titrate the excess  $\text{AgNO}_3$  with standard  $\text{NH}_4\text{CNS}$  to the first definite end point.

‡ A 15 ml. Gooch crucible with a rapid filtering mat, ca. 3 mm. thick, of acid-washed, long-fibered asbestos, to which is added 0.5–1 ml. of  $\text{CaCO}_3$  suspension in such manner that the asbestos is completely covered when the liquid is removed by suction. The asbestos pad may be used for 3 or 4 determinations. The top surface, impregnated with platinum powder, may be removed with a sharp wire when filtering becomes too slow, and a thin layer of asbestos may be added from time to time if pad becomes thin. The asbestos should be completely covered with a layer of  $\text{CaCO}_3$  before each filtration.

§ If a furnace is not available, the ignition is conveniently conducted as follows: Prepare an air bath by suspending a nichrome triangle ca. 1 inch from the bottom of a metal crucible ca. 2.5 inches in diameter at the top and 3 inches deep and placing an inverted porcelain crucible cover on the triangle. Heat over a Meker burner with the flame so adjusted that the inside of the crucible is just red as far up as the suspended cover.

filter by suction into a 400–500 ml. glass-stoppered Erlenmeyer. Repeat the addition of hot water once or twice to remove the  $\text{Na}_2\text{CO}_3$ . With suction on, add the  $\text{HNO}_3$  (ca. 5 ml.) to the Gooch dropwise to decompose the  $\text{CaCO}_3$  and  $\text{Na}_2\text{CO}_3$ . Wash several times with hot water.

Add 10 ml. of the  $\text{HNO}_3$  to the flask. Cool, and add 5 ml. of the ferric alum and a quantity of the standard  $\text{AgNO}_3$  solution, accurately measured, greater than that necessary to precipitate the chloride. Make to a volume of ca. 200 ml. with water, add 1–2 ml. of nitrobenzene, stopper the flask, and shake vigorously for ca. 30 seconds to coagulate the  $\text{AgCl}$ . Titrate the excess  $\text{AgNO}_3$  with the  $\text{KCNS}$ . 1 ml. of 0.1  $N$   $\text{AgNO}_3$  = 8.1 mg. of  $\text{K}_2\text{PtCl}_6$ , 1.57 mg. of  $\text{K}_2\text{O}$ , or 1.3 mg. of  $\text{K}$ .

If the approximate  $\text{AgNO}_3$  requirement is not known, the following procedure is recommended: After adding the ferric alum, note the reading on the  $\text{KCNS}$  buret and add a few drops to the flask. Then, while swirling the flask, run in the standard  $\text{AgNO}_3$  from a buret until the solution becomes milk white, after which add an additional 1 or 2 ml. Continue the determination as directed previously, beginning "make to a volume of ca. 200 ml. . . ." Back titrate with the  $\text{KCNS}$  and include the volume added previous to the addition of  $\text{AgNO}_3$  in calculating the  $\text{AgNO}_3$  equivalent of the sample.

#### Cobaltinitrite Method

#### 4

##### REAGENTS

(a) *Trisodium cobaltinitrite solution*.—Prepare an aqueous solution containing 2.0 grams of the salt reagent specially prepared for potassium tests in each 10 ml. Filter before use and prepare fresh solution before each set of determinations.

(b) *Nitric acid solutions*.—Approximately 1  $N$  and 0.01  $N$ .

(c) *Nitric acid—Dipotassium sodium cobaltinitrite wash solution*.—Saturate a portion of the 0.01  $N$   $\text{HNO}_3$  with a few mg. of  $\text{K}_2\text{NaCo}(\text{NO}_2)_6 \cdot \text{H}_2\text{O}$  by shaking (ca. an hour). Filter through an F Pyrex sintered-glass crucible or equivalent.

#### 5

##### DETERMINATION

Add enough 1  $N$   $\text{HNO}_3$  to the ash, 1 (a) or 1 (b) in a platinum dish to yield an excess of ca. 2 ml. of the acid in the aliquot taken for precipitation (ca. 3 ml.). Wash into a small volumetric flask (25 ml.), make to volume, and mix. Allow to stand at least 1 hour and filter, if necessary, through a small paper filter. Withdraw a 10 or 20 ml. aliquot (3–35 mg. of  $\text{K}_2\text{O}$ ), adjust to 20 ml. if necessary, and cool to ca. 20°.

Add from a pipet while stirring 10 ml. of the sodium cobaltinitrite solution cooled to 20°C. In the range 3–18 mg. (most preserves) add the reagent dropwise with stirring; in the range 18–35 mg. (most fruits) add the reagent in a steady stream from a fairly rapid delivery pipet (20–22 seconds). Allow to stand 2 hours at ca. 20°. Protect precipitating vessel from laboratory fumes by placing under bell jar or similar device. Filter in a tared sintered-glass filtering crucible (Pyrex F 35 ml. capacity is convenient), using the saturated cobaltinitrite solution in 0.01  $N$   $\text{HNO}_3$  to make transfer.

Wash precipitate nine times with at least 4 ml. portions of this solution, once with 2 ml. of 0.01  $N$   $\text{HNO}_3$ , and 5 times with 2 ml. portions of alcohol, releasing vacuum each time before adding the washing fluid. Aspirate until quite dry. Dry for 1 hour at 100°, cool in desiccator, and weigh. The formula of the precipitate is  $\text{K}_2\text{NaCo}(\text{NO}_2)_6 \cdot \text{H}_2\text{O}$ , and

$$\frac{\text{mg. of precipitate} \times 0.20738 \times 100}{\text{g. of sample in aliquot}} = \text{mg. of } \text{K}_2\text{O} / 100 \text{ g. sample.}$$

## NOTES:

(1) *Crucibles*.—Pyrex, F porosity, have been found acceptable as have the Jena G<sub>4</sub> crucibles. Either can be used a number of times before the H<sub>2</sub>SO<sub>4</sub> cleaning treatment by dusting out the weighed cobaltinitrite precipitate. The complete cleaning is necessary only when pores show evidence of clogging.

(2) *Washing of precipitate*.—The volumes of the saturated wash liquor are not important but the final wash with 0.01 N HNO<sub>3</sub> should be restricted to 2 ml. The K<sub>2</sub>NaCo(NO<sub>2</sub>)<sub>6</sub> precipitate obtained in potassium determinations is suitable for saturating the wash solution.

(3) *Control sample*.—A control sample of pure dry KCl should be run from time to time. A stock solution of 2 mg. of K<sub>2</sub>O per ml. is convenient. Adjust to a volume of 20 ml., using 2 ml. of 1 N HNO<sub>3</sub> for acidification. If recoveries are low, the reagent should be rejected; if slightly high, a blank correction may be made on the potassium estimations.

(4) The potassium cobaltinitrite is conveniently removed from the crucible by dissolving in hot 5% by volume H<sub>2</sub>SO<sub>4</sub> in water.

P<sub>2</sub>O<sub>5</sub> IN FRUITS AND FRUIT PRODUCTS

Associate Referee Shuman subjected the revised volumetric procedure for P<sub>2</sub>O<sub>5</sub> in fruit products to collaborative study, and his report contains results from the analysis of three fruit samples analyzed by ten collaborators. The data indicate excellent agreement between the volumetric and the official colorimetric methods.

## COLD-PACK FRUITS

The associate referee is attacking the important problem of sampling cold-pack fruit. The preliminary report indicates progress in the design and use of equipment for sampling large containers.

## POLARISCOPIC METHODS

The associate referee has submitted no report, but in correspondence he has indicated progress in studies on the determination of the effect of pectin in polariscopic determinations. These studies will be continued.

## ELECTROMETRIC TITRATION OF ACIDITY

While no report has been submitted, the associate referee in correspondence has outlined additional work on this subject, which studies are now in progress.

## SODIUM AND CHLORIDES

No report was received from the associate referee. In a contributed paper (see p. 437) the referee shows the necessity for the addition of sodium carbonate as a fixative for chlorine during ashing of all fruit samples that contain significant amounts of chlorides.

## ORGANIC ACIDS

The reader is referred to the excellent contribution of B. G. Hartmann on the polybasic acids of fruits and fruit products, which appears in this issue of *The Journal* (see p. 444).



## REPORT ON POTASSIUM IN FRUITS AND FRUIT PRODUCTS

### VOLUMETRIC CHLOROPLATINATE METHOD

By HAROLD W. GERRITZ (U. S. Food and Drug Administration,  
San Francisco, Calif.), *Associate Referee*

Last year a volumetric chloroplatinate method for the determination of potassium adaptable to control work in jam and jelly plants was presented (*This Journal*, 25, 232). Later (*Ibid.*, 433) results of a collaborative study of the method were reported. The method was subjected to further collaborative study this year.

The following instructions were furnished collaborators:

Last year the method was applied collaboratively to homogeneous samples (sample solution). The samples this year are fruits heterogeneous in nature. Sample 1 is blackberry, Sample 2 is loganberry, and Sample 3 is apricot. The samples are preserved with formaldehyde. Mix all samples well while removing portion for analysis.

Ash 15-16 grams of accurately weighed sample in duplicate, according to "Preparation of Sample (b)" (*Ibid.*, 234) and determine  $K_2O$  by the method there described—with the following exception: Beginning with the first sentence on page 235 substitute for the remainder of the paragraph "wash crucible free of soluble chloroplatinates with 90% alcohol. Cover the  $K_2PtCl_6$  completely with the  $Ca(CHO_2)_2$  by adding ca. 1 ml. of the suspension. Remove the liquid with suction and wash once with alcohol. Remove crucible from filter attachment. Wet the residue with 0.5-1 ml. of alcoholic NaOH, cover with  $Na_2CO_3$  to a depth of 2-3mm., and ignite at ca. 500° C. for 5-10 minutes."

The asbestos pad lasts for 5-10 determinations, and filtration is rapid if the carbon is burned out from time to time. Beginning with the first sentence of the footnote on page 235, the following should be substituted for the remainder of the paragraph: "The asbestos pad lasts for 5-10 determinations. After 2 or 3 determinations, heat the crucible to redness over a flame to dispel the carbon."

The crucibles tend to clog during the filtration of the chloroplatinate from some samples. This is true of Sample 2, of this series. The filtration may then be speeded by allowing air to be sucked through the filter until the sludge on the asbestos becomes dry and cracks like drying clay.

For the precipitation of the chlorides 30 ml. of 0.1 N  $AgNO_3$  may be added for Sample 1, 20 ml. for Sample 2, and 50 ml. for Sample 3.

Report results as mg. of  $K_2O$  per 100 grams of sample.

Samples were sent to a number of collaborators from whom reports have not been received. Results from those reporting are presented in Table 1.

TABLE 1.—*Collaborative results*  
(mg. of  $K_2O$ /100 grams of sample)

COLLABORATOR*	SAMPLE 1	SAMPLE 2	SAMPLE 3
Daniel Banes	248.8-251.2	148.3-148.6	419.0-416.6
R. H. Dick	258.3-257.5	147.2-146.8	425.7-425.8
H. W. Gerritz	256.1-255.9	148.0-147.8	427.7-427.4
D. W. Williams	260.8-258.6	146.7-147.6	429.1-429.9
C. A. Wood	245.5-247.5	147.2-146.9	413.3-418.7

\* All collaborators are employed by the U. S. Food and Drug Administration.

It will be noted from Table 1 that results on Samples 1 and 3 by Analysts 1 and 5 are somewhat lower than the results reported by other analysts. It has been found by experience that ignition much above 500°C.

TABLE 2.—*Results by volumetric and official methods submitted by R. H. Dick (mg. K<sub>2</sub>O/100 grams of sample)*

MATERIAL	VOLUMETRIC METHOD	OFFICIAL METHOD	MATERIAL	VOLUMETRIC METHOD	OFFICIAL METHOD
Blackberries	137.7	136.1	Grape jelly	131.1	132.8
Blackberries	192.6	190.0	Grape jelly	110.4	111.0
Blackberries	186.8	186.3	Grape jelly	133.5	135.0
Blackberries	186.8	185.6	Grape jelly	123.3	123.6
Blackberries	181.4	182.1	Loganberry jelly juice	200.8	200.5
Blackberries	181.5	181.6	Loganberry jam	115.8	115.1
Blackberries	186.6	187.9	Loganberry jam	103.7	102.4
Blackberries	199.3	198.4	Peaches	204.1	203.7
Blackberries	197.5	196.1	Peaches	199.7	198.2
Blackberries	181.9	177.5	Peach jam	100.4	100.6
Blackberries	182.0	181.4	Peach jam	98.1	98.2
Blackberries	204.8	202.4	Plums	188.0	192.1
Blackberries	216.3	212.6	Plums	123.1	124.3
Blackberries	196.6	193.8	Plums	180.6	175.7
Blackberries	188.3	185.0	Plums	145.0	145.1
Blackberries	182.7	180.6	Plum jelly juice	261.7	259.0
Blackberries	177.6	177.0	Plum jam	84.6	84.5
Blackberry jelly juice	172.0	168.1	Plum jam	117.4	116.3
Blackberry jelly juice	251.3	248.0	Quince	129.5	131.4
Blackberry jelly	81.1	79.7	Quince	150.1	148.8
Blackberry jelly	136.4	135.8	Quince jelly juice	117.2	118.3
Crabapples	247.9	251.1	Quince jelly juice	290.8	289.6
Crabapple jelly juice	160.8	160.3	Quince jelly	115.7	117.5
Crabapple jelly	237.3	236.5	Quince jam	127.0	123.0
Currant jelly juice	343.0	340.4	Raspberries	108.6	105.9
Currant jelly juice	346.8	345.5	Raspbèrry jelly juice	255.1	254.6
Currant jelly	236.2	236.0	Raspberrry jelly juice	139.3	139.8
Grapes	217.4	217.1	Raspberrry jelly	119.0	119.1
Grapes	308.6	312.6	Raspberrry jelly	107.4	104.8
Grape jelly juice	212.0	213.8	Strawberries	123.9	124.0
Grape jelly juice	184.7	182.5	Strawberries	137.0	136.9
Grape jelly juice	226.2	227.7	Strawberry jam	108.6	108.2
Grape jelly juice	192.0	194.0			

will often cause a loss of chlorides from large charges of potassium chloroplatinate. The differences shown between duplicate results on the samples referred to above lead the Associate Referee to believe that chlorides were lost during ignition.

In addition to collaborative data, R. H Dick submitted results on a number of samples that he analyzed by the volumetric method referred to and by the official gravimetric method. The samples consisted of water-packed fruits, jelly juices, jellies, and jams. These data are reported in Table 2.

### CONCLUSION

The data presented, together with collaborative data presented at the last meeting, indicate that the method is capable of accurate results.

## REPORT ON $P_2O_5$ IN FRUITS AND FRUIT PRODUCTS

### VOLUMETRIC METHOD

By HARRY SHUMAN (Food and Drug Administration, Federal Security Agency, Philadelphia, Pa.), *Associate Referee*

In accordance with the recommendation of last year, the proposed volumetric method was studied collaboratively in comparison with the colorimetric method.

Sample solutions containing 15 grams of the product in 100 ml. were prepared from (1) apricot jam, (2) currant jelly, and (3) raspberry jam. Solutions were preserved with formaldehyde (0.5 ml. 40% HCHO in 100 ml. of solution).

Collaborators were requested to determine  $P_2O_5$  by using the volumetric procedure and molybdate reagent proposed by the writer (*This Journal*, 25, 437) and the colorimetric method (*Methods of Analysis*, A.O.A.C., 1940, 347). For the colorimetric method, collaborators were given the option of employing wet digestion or ashing. Directions for ashing were as follows:

Obtain the ash, using 50 ml. of the prepared solution. Add to the ash 10–15 ml. of water and 3 or 4 ml. of HCl and evaporate to dryness on the steam bath. Take up in 5 ml. of hot HCl (1+9), transfer to a 100 ml. volumetric flask, cool, and make to volume. Proceed as directed in *Methods of Analysis*, A.O.A.C., 1940, page 348, paragraph 41, using 20 ml. aliquots.

Table 1 shows that the results obtained by the proposed volumetric method are in good agreement with results by the official colorimetric method.

TABLE 1.—*Collaborative results*  
(mg./100 grams of sample)

COLLABORATOR†	SAMPLE 1 APRICOT		SAMPLE 2 CURRANT		SAMPLE 3 RASPBERRY		BLANK (0.1 N NaOH)
	V*	C†	V*	C†	V*	C†	V*
J. L. Hogan, New York	21.7	21.8	17.1	17.4	13.4	13.8	—
	21.7	21.7	17.0	17.5	13.3	13.8	—
D. W. Williams, San Francisco	21.7	22.0	17.2	17.5	13.2	13.5	0.16
	21.9	22.0	17.2	17.5	13.4	13.6	0.16
M. Tubis, Philadelphia	21.7	22.4	17.5	16.7	13.5	13.7	0.02
	22.2	22.7	17.3	16.7	13.8	13.7	0.02
H. Shuman, Philadelphia	21.9	21.6	17.3	16.9	13.3	13.1	0.04
	22.1	22.0	17.3	16.9	13.5	13.3	0.04
H. W. Gerritz, San Francisco	21.7	22.1	17.3	17.2	13.4	13.4	0.01
	21.8	22.1	—	17.3	—	13.6	0.02
J. F. Weeks, Jr., New Orleans	22.0	22.0	17.0	17.0	13.0	13.0	0.00
	—	—	—	—	—	—	0.00
J. W. Sanders, Jr., Atlanta	22.0	22.0	17.5	16.5	14.0	13.3	0.05
	21.8	22.2	17.6	16.8	13.9	13.3	0.10
	21.9	21.7	17.5	17.9	13.9	13.3	—
D. Banes, Chicago	21.6	21.7	16.8	17.0	12.9	12.9	0.12
	22.0	22.0	16.3	17.1	12.7	12.7	0.26
D. Miller, Buffalo	22.3	22.7	17.5	18.0	13.5	13.3	0.05
	22.3	—	17.5	—	13.4	—	0.05
R. A. Osborn, Washington	22.0	21.3	17.3	16.7	13.1	12.4	0.18
	22.2	21.8	17.5	16.8	13.1	13.0	0.21
Max.	22.3	22.7	17.6	18.0	14.0	13.8	—
Av.	21.9	22.0	17.2	17.2	13.3	13.3	—
Min.	21.6	21.3	16.3	16.5	12.7	12.4	—

\* Volumetric method.

† Colorimetric method.

‡ All collaborators are members of the U. S. Food &amp; Drug Administration.

## REPORT ON SAMPLING COLD-PACK FRUIT

By PAUL A. MILLS (Food and Drug Administration, Federal  
Security Agency, Seattle, Wash.), *Associate Referee*

An attempt was made to devise a means of sampling large packages of frozen fruit. Previous procedures, which required extensive equipment,

consisted of thawing the package, draining, and taking proportionate samples of the solid and liquid.

A large hollow trier, 2 inches in diameter and 30 inches long, was de-

TABLE 1.—*Trier sampling of barrels*

(Average composition of barrel frozen fruit (composite of 8 trier segments) compared with corresponding composition of fresh fruit)

	AS IS BASIS		SUGAR-FREE BASIS—			
	SOLUBLE SOLIDS REFRACT. 20°C.	TOTAL SUGARS AS INVERT	INSOLUBLE SOLIDS	NON-SUGAR SOLIDS	ASH	ACID AS CITRIC
	per cent	per cent	per cent	per cent	per cent	per cent
Straight frozen blackber- ries from barrel	11.37	8.58	6.25	3.05	.412	.535
	av.					
Same lot blackberries fresh fruit sample	10.80	7.68	5.69	3.38	.406	.656
Straight frozen strawber- ries	8.68	6.37	2.26	2.47	.346	.721
Same lot strawberries fresh fruit sample	8.53	6.36	1.99	2.32	.341	—
Frozen strawberries and sugar from barrel (5 fruit to 1 sugar)	23.98	22.21	2.35	2.28	.381	.738
Same lot strawberries fresh fruit sample	8.80	6.47	2.06	2.49	.357	—
Strawberries frozen from barrel (3 fruit 1 sugar)	29.37	28.19	2.77	1.64	.379	.723
Same lot strawberries fresh fruit sample	8.47	6.40	2.03	2.21	.349	—

signed for sampling material in the frozen state (see Figures 1, 2, and 3). This trier was made of 18 gage Shelby steel tubing. Large saw teeth were cut into one end and so set as to cut a hole large enough to allow the tube to run freely and at the same time cut the core large enough to fit tightly in the tube. The other end was fitted with an easily removable handle for rotating the tube. After insertion, a No. 11 rubber stopper placed in the handle end allows the removal of the tube with its contents. The contents can then be forced out with a wooden ram. With materials at low tem-

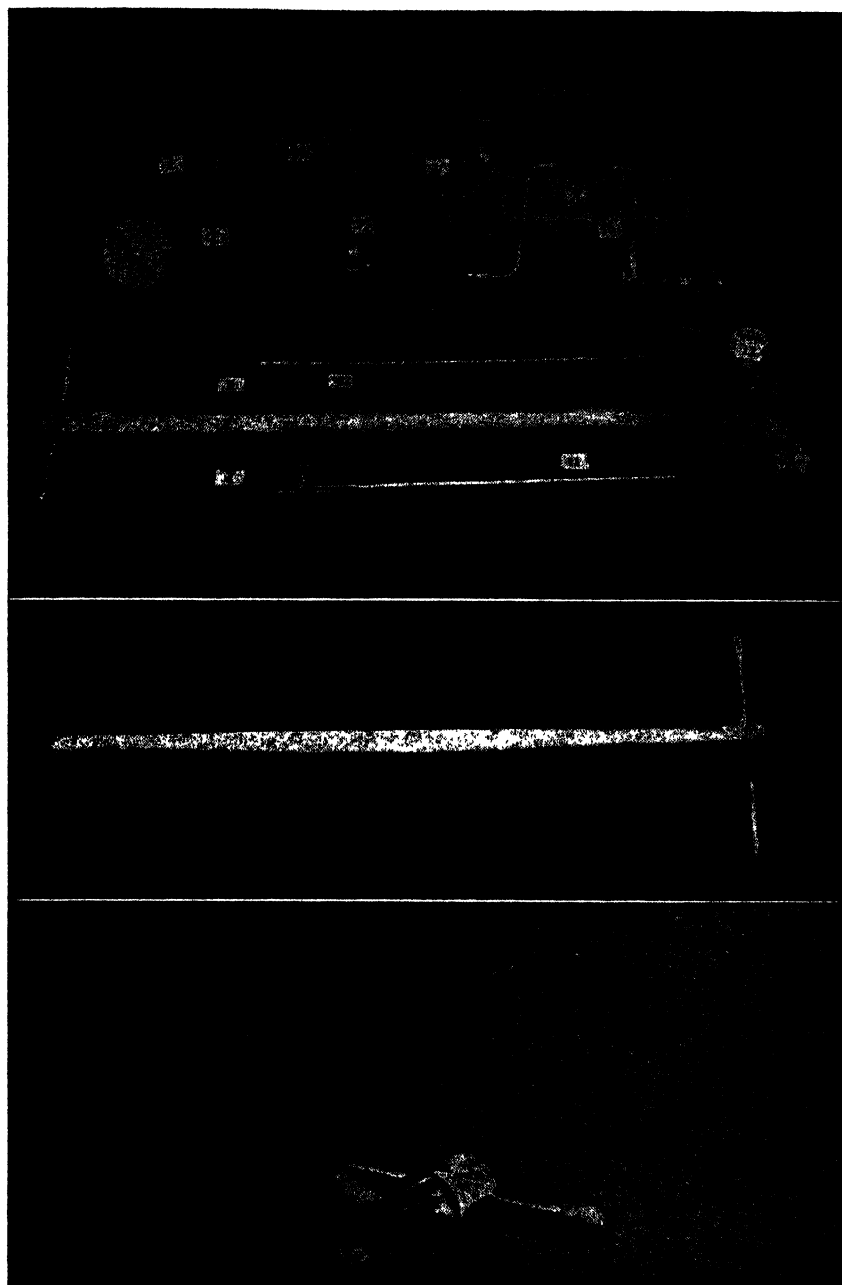


FIG. 1 (Top).—Inspector's tool kit containing frozen fruit trier and barrel bungs. Designed by Eric A. Gray, Chief Inspector, Seattle Station, 1938.

FIG. 2 (Center).—Frozen fruit trier.

FIG. 3 (Bottom).—Close-up of cutter and handle.

peratures ( $-10^{\circ}$  to  $0^{\circ}\text{F.}$ ), the core often freezes in the trier and it is necessary to ram out the section and continue drilling in the same hole.

Three barrels of strawberries, one packed without sugar, and two packed with varying quantities, and one barrel of blackberries without sugar were prepared under supervision in a commercial packing plant. At the time of packing, samples of the fruit consisting of 12 quarts to represent each barrel were taken for analysis with the intention of treating them as master samples of the fruit used. After freezing, the barrels were held in commercial storage and finally sampled as follows:

Five full cores ca. 26 inches long were taken from the top of the barrel

*Diagram of Sampling.*

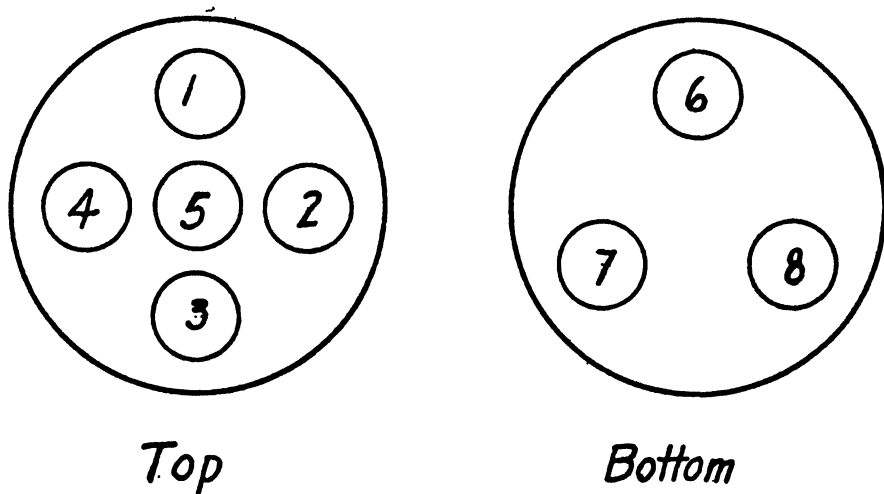


FIG. 4.—Arrangement of cores taken from barrel.

and three from the bottom in the manner illustrated by the diagrams. These cores reached to within ca. 4 inches of the bottom of the barrel.

The samples of fruit taken at the time of packing were analyzed immediately, and the samples of the frozen barrels were held in cold storage until analysis could be made. Each entire separate core was analyzed separately, and average results are given in the table.

In the case of the blackberries, it is apparent that the soluble solids and sugars in the frozen barrels are higher than those in the sample of fresh fruit. This indicates that the ripening process continues during freezing and possibly in storage. The ash is affected very little and is apparently rather well distributed throughout the barrel. Total acid as citric is also well distributed, while sugars, soluble solids, and insoluble solids show considerable variation from core to core.

With strawberries, the pack without sugar shows a picture similar to the blackberries, except that there is not so much difference between the

results for the fresh fruit sample and the average results of the samples of frozen fruit.

In the sugar packs, bleeding of the strawberries was apparent. There was about 4 inches of practically fruitless frozen sirup in the bottom of the 5+1 barrel, and roughly 12 inches in the bottom of the 3+1 barrel.

The barrel of 3+1 strawberries and sugar, which was stored at 12°-15°F., was not hard-frozen although it was quite firm. The sirup in the bottom of the barrel could be cut, but it would flow or settle slowly. In the case of several cores, part of the sirup that was at the bottom was not retained well by the trier. In the 5+1 barrel, which was frozen harder on account of a lower sugar concentration, two cores probably did not include all the sirup in the bottom of the barrel because the barrels were initially frozen on their sides (in this case 48 hours at 0°F.) and there was a separation of berries and juice from one side to the other as well as from top to bottom. It was noted also that in both sugar packs the center of the barrels, represented by one core, was not so hard-frozen as were those parts toward the sides. This fact indicates that freezing is not complete during the initial freeze and proceeds during storage.

It is recommended that this study be continued. Consideration should be given to the construction of a new trier of stainless steel tubing, one inch inside diameter and 40 inches long, fitted with removable cap enabling the use of an electric drill. Large saw teeth should be filed into the cutting end with sufficient set to allow the trier to run free. The reason for the smaller bore is the greater certainty that the core is retained in the trier when it is withdrawn. The greater length should make sampling possible through the entire length or diagonal length of the barrel. Smaller cores will also allow complete sampling without so much damage to the package and will avoid the collection of an excessive quantity of material. Stainless steel, rather than tinned steel, is recommended because of its smoothness, which especially facilitates removal of the core.

The fruit representing each barrel should consist of a relatively large number of subdivisions, for example, 36 quarts. These should be treated and stored exactly like the barrel so far as possible, and jars should be withdrawn and analyzed weekly or twice weekly in order to trace the suspected ripening after packing. The analysis should include soluble solids, insoluble solids, acidity, ash, phosphorus ( $P_2O_5$ ), and potassium ( $K_2O$ ). The master sample should be analyzed after the storage period instead of immediately, as was done this year. Storage conditions for this sample should approximate those for the barrel.

In addition to repeating this year's work, it may be advisable to prepare barrels containing added water. Probably two such barrels, one containing 10 per cent added water and the other 20 per cent, should be enough for preliminary work.



## REPORT ON FAT IN COOKED ANIMAL FEEDS CONTAINING CEREALS (ACID HYDROLYSIS)

By STACY B. RANDLE (Kentucky Agricultural Experiment Station,  
Lexington, Ky.), *Associate Referee*

Last year the Associate Referee (*This Journal*, 25, 864) submitted a report on this project. This year's investigation was limited to a study of the fat content of dog feeds by the direct ether extraction method (Bailey-Walker) and by the acid hydrolysis method. Some of the disadvantages of the acid hydrolysis method were stated in the earlier report, and therefore will not be discussed at any length here.

The Association recommended that further investigational studies be made on this problem and also that collaborative studies be conducted. This has been done, but owing to excessive war work some difficulty was experienced in obtaining collaborators. Several chemists were unable to complete the work after receiving the samples.

### EXPERIMENTAL DATA

This investigation is based on the official method for the determination of fat in wheat flour described on page 213, *Methods of Analysis, A.O.A.C.*, 1940.

#### FAT (ACID HYDROLYSIS METHOD)

Place 2 g. of the flour in 50 ml. beaker, add 2 ml. of alcohol, and stir so as to moisten all particles. (Moistening of samples with alcohol prevents lumping on addition of the acid.) Add 10 ml. of HCl (25+11), mix well, set beaker in water bath held at 70–80°, and stir at frequent intervals 30–40 min. Add 10 ml. of alcohol and cool. Transfer mixture to Röhrig or Mojonnier fat extraction apparatus. Rinse beaker into extraction tube with 25 ml. of ethyl ether in 3 portions and shake mixture well. Add 25 ml. of redistilled petroleum benzin (b.p. below 60°) and mix well. Let stand until upper liquid is practically clear. Draw off as much as possible of ether-fat solution through filter consisting of pledget of cotton packed just firmly enough in stem of funnel to allow free passage of ether into weighed 125 ml. beaker-flask containing some porcelain chips or broken glass. Before weighing beaker-flask, dry it and a similar flask as a counterpoise in drying oven at 100° and then allow it to stand in air to constant weight. Re-extract liquid remaining in tube twice, each time with only 15 ml. of each ether. Shake well on addition of each ether. Draw off clear ether solutions through filter into same flask as before and wash tip of spigot, funnel, and end of funnel stem with a few ml. of a mixture of the 2 ethers in equal volumes free from suspended H<sub>2</sub>O. Evaporate ethers slowly on steam bath, then dry fat in drying oven at 100° to constant weight (ca. 90 min.). Remove flask and counterpoise from oven, allow to stand in air to constant weight (ca. 30 min.), and weigh. (Owing to size of flask and nature of the material, there is less error by cooling in air than in desiccator.) Correct this weight by blank determination on reagents used. Report as per cent fat by acid hydrolysis.

The Associate Referee used Röhrig tubes exclusively for these studies. Mallinckrodt's petroleum benzin, analytical reagent (b.p. 30°–60°C.), was found to be satisfactory without redistillation, and 150 ml. Griffin beakers

were more convenient to use than was the 125 ml. beaker-flask. Satisfactory results were obtained without the use of porcelain chips or broken glass.

For the collaborative study seven samples of dog feed, three of which were canned samples that had been dried, were distributed. All canned samples were prepared as previously described in *This Journal*, 25, 864. The results of collaborators are shown in Table 1. Throughout this paper, the official fat method will be abbreviated to BW and the acid hydrolysis method to AH.

TABLE 1.—*Collaborative results*  
(Percentage of fat extracted by Bailey-Walker and acid hydrolysis methods)

COLLABORATOR	SAMPLE 1*		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5		SAMPLE 6		SAMPLE 7	
	BW†	AH‡	BW	AH	BW	AH	BW	AH	BW	AH	BW	AH	BW	AH
Budde	10.80	12.90	8.50	10.90	14.20	15.90	5.20	6.60	6.40	7.90	3.60	5.80	2.60	4.60
Doty	11.60	13.44	8.95	11.72	15.10	16.21	5.57	7.19	6.55	8.64	4.13	7.20	3.00	4.90
Maroney	10.90	12.60	8.70	10.75	14.70	16.05	5.05	6.65	6.55	8.00	3.70	5.85	2.90	4.80
Randle	11.44	11.61	8.85	10.19	14.64	14.47	5.35	5.84	6.60	7.34	3.93	5.44	2.79	4.49
Average	11.19	12.64	8.75	10.89	14.66	15.66	5.29	6.57	6.53	7.97	3.84	6.07	2.83	4.70
Range	0.80	1.83	0.45	1.53	0.90	1.74	0.37	1.35	0.20	1.30	0.53	1.76	0.40	0.41

\* Samples 1, 2, and 3 were canned dog feed, and the percentage fat in these samples is expressed on the dry basis. The percentage fat in the other samples is expressed on the air-dried sample.

† Official fat method.

‡ Acid hydrolysis method.

Table 1 shows clearly that this study should be continued with more analysts participating. In general, the analysts are in closer agreement by the Bailey-Walker method than by the acid hydrolysis method. However, in some cases there are wide variations between analysts on both methods, although some checks are very close. With the exception of Sample 7, the differences between the low and high results are much greater for the acid hydrolysis than for the Bailey-Walker method. Generally, it is possible to obtain good checks by the Bailey-Walker method, but in some cases it is very difficult to get close checks by the acid hydrolysis method. The acid hydrolysis method is more tedious and requires much greater skill in operation than does the direct ether method.

#### COLLABORATORS

E. F. Budde, Quaker Oats Company, Chicago, Ill.

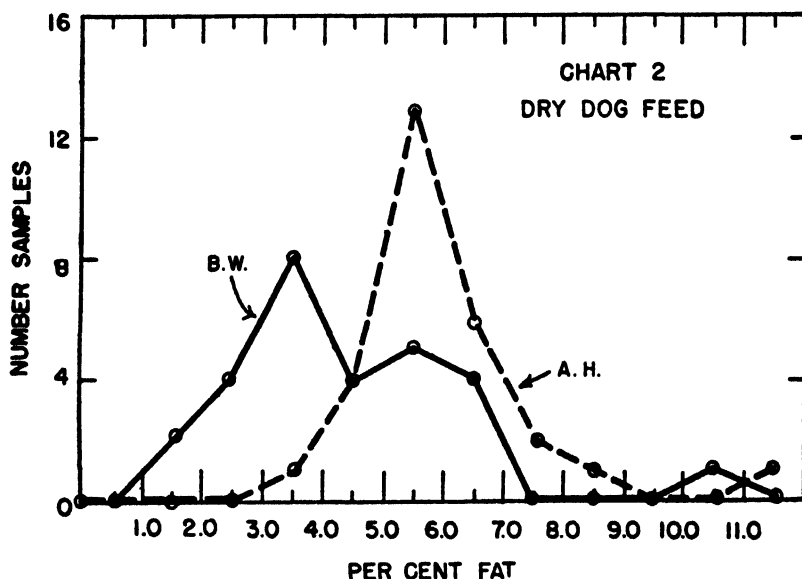
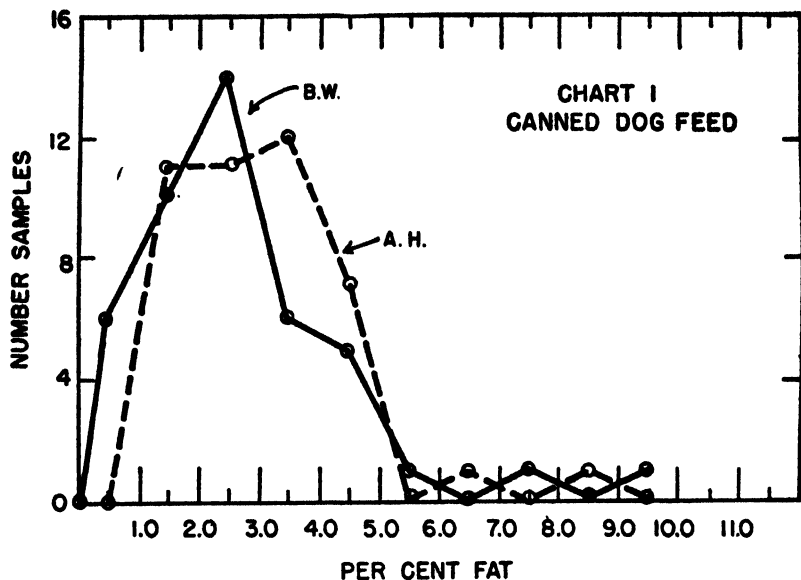
D. M. Doty, Purdue University, Lafayette, Ind.

J. E. Maroney, American Meat Institute, Chicago, Ill.

Stacy B. Randle.

#### ADDITIONAL EXPERIMENTAL DATA

In order to study this problem further, the Associate Referee analyzed 71 samples of dog feeds by the acid hydrolysis and the Bailey-Walker methods. Forty-three of these samples were canned dog feed and the remaining 28 were dried dog feed. All figures given in these studies are averages of at least two determinations.



The average fat content of the canned dog feed by the Bailey-Walker method was 2.68 per cent, while the average for the same samples by the acid hydrolysis method was 3.07 per cent (Table 2). These samples were dried as previously described, and the percentage fat was calculated to the original canned feed. Chart 1 shows graphically the frequency distribution for canned dog feeds by the two methods. The acid hydrolysis curve does not give a distinct peak as does the Bailey-Walker curve. The peak for

TABLE 2.—*Comparison of fat content of dry and canned dog feed by two methods*

LAB. NO.	B.W.	A.H.	LAB. NO.	B.W.	A.H.
Dry dog feed					
2084	1.29	3.49	1513	4.08	5.59
1514	1.84	4.94	1515	4.21	5.92
1511	2.02	4.35	1713	4.26	5.19
1677	2.70	5.42	1525	4.29	5.62
1626	2.79	4.49	1734	5.10	6.10
1671	2.95	5.47	1753	5.10	6.08
1679	3.12	6.42	1623	5.35	5.84
1529	3.23	5.37	1714	5.50	6.47
1526	3.32	5.63	1646	5.88	6.43
1733	3.40	5.34	1752	6.00	6.29
1776	3.62	4.03	1758	6.20	7.16
1711	3.74	5.78	1624	6.60	7.34
1756	3.87	5.17	1712	6.88	8.17
1625	3.93	5.44	1631	10.92	11.68
Average				4.36	5.90

LAB. NO.	B.W.	A.H.	LAB. NO.	B.W.	A.H.	LAB. NO.	B.W.	A.H.
Canned dog feed								
1635	0.46	1.11	1760	1.91	2.25	1487	3.05	3.37
1523	0.51	1.04	1754	2.03	2.43	1645	3.18	3.51
1512	0.74	2.55	1755	2.08	2.49	1672	3.33	3.43
1670	0.75	1.42	1762	2.27	2.73	1478	3.46	3.51
1647	0.92	1.61	1629	2.46	2.77	1628	3.77	4.27
1527	0.98	1.54	1510	2.55	2.96	1761	3.88	4.05
1640	1.00	1.46	1639	2.64	3.70	1637	4.18	4.46
1633	1.17	1.64	1644	2.67	3.08	1674	4.32	4.30
1642	1.18	1.95	1522	2.75	2.70	1675	4.40	4.52
1632	1.21	1.82	1643	2.80	3.17	1498	4.77	4.72
1638	1.28	1.73	1757	2.82	3.26	1676	5.41	4.92
1630	1.39	1.94	1521	2.82	3.07	1528	7.23	6.91
1641	1.40	2.22	1524	2.88	3.11	1627	9.16	8.93
1669	1.79	2.23	1636	2.90	3.41			
1673	1.87	2.16	1759	2.98	3.52			
Average							2.68	3.07

the acid hydrolysis method shows one per cent more fat than does that for the direct method. Since the Kentucky standard for minimum fat in canned dog feeds is 2 per cent, Chart 1 shows that 16 samples failed to meet this guarantee by the direct method whereas 11 samples failed by the acid hydrolysis method.

For the dry dog feed samples, the direct method gave an average of 4.36 per cent fat and the acid hydrolysis method an average of 5.90 per cent fat. Chart 2 for the dry dog feeds shows that the peak for the acid hy-

drolysis method is 2 per cent more fat than for the direct method. Since Kentucky has no minimum fat standard for dry dog feeds, a comparison of those failing to meet the guarantee cannot be made, as in the case of canned dog feeds.

An attempt is being made to investigate the materials extracted by the two methods. It was thought advisable to extract samples of dog feed by the direct ether method and then apply the acid hydrolysis method to the extracted residue. This procedure was applied to ten samples of dog feed, and the results are recorded in Table 3. These analyses were made several weeks later than those given in Table 2. Any differences in fat content of samples in Tables 2 and 3 are probably due to changes in the

TABLE 3.—*Acid hydrolysis of residue after Bailey-Walker extraction*

SAMPLE NO.	BAILEY-WALKER EXTRACTION (1)	ACID HYDROLYSIS AFTER BAILEY-WALKER EXTRACTION (2)	SUM OF COLUMNS 1 & 2 (3)	ACID HYDROLYSIS (4)
1623	5.37	2.09	7.46	5.84
1624	6.62	1.75	8.37	7.34
1625	3.97	2.57	6.54	5.44
1712	6.77	1.70	8.47	8.17
1713	4.29	1.77	6.06	5.19
1733	3.64	1.74	5.38	5.34
1754	2.04	0.61	2.65	2.43
1641	1.36	0.78	2.14	2.22
1677	2.77	2.48	5.25	5.42
1679	2.95	2.32	5.27	6.42
Average	3.98	1.78	5.76	5.38

moisture content of the sample in the meantime. The moisture was not determined at the time the analyses shown in Table 3 were made.

Column 1 shows the per cent fat extracted by the direct ether method and Column 2 the fat removed by the acid hydrolysis method after direct ether extraction; Column 3 is the sum of Columns 1 and 2. Column 4 gives the per cent fat obtained by the acid hydrolysis method. If it is assumed that the Bailey-Walker method does not extract all the fat from a sample and that the acid hydrolysis method does, then Column 3, which is the sum of Columns 1 and 2, and Column 4 should agree very closely. This is true for some samples. However, the average for Column 3 is 0.38 per cent more fat than that for Column 4. It is generally agreed that the Bailey-Walker method measures crude fat and that this method extracts materials other than true fats. Then it appears by inference from this preliminary investigation that the acid hydrolysis method also removes materials other than true fats. The Associate Referee considers that this investigation should be extended to a study of the materials removed by the two methods.

Since one disadvantage of the acid hydrolysis method is the formation of emulsions and the separation of the extraneous material from the fat, it was thought that use of weaker acid might be an improvement. In the acid hydrolysis method the proportion of concentrated hydrochloric acid to water is 25 to 11. This ratio was reversed, i.e. changed to 11 ml. of acid and 25 ml. of water, and 19 samples were analyzed by the weaker acid. Table 4 compares the results obtained by the use of the stronger and

TABLE 4.—*Acid hydrolysis of dog feeds—two concentrations of acid compared*

LAB. NO.	STRONG ACID	WEAK ACID	DIFFERENCE
1498	4.72	5.15	+0.43
1514	4.94	4.73	-0.21
1524	3.11	3.19	+0.08
1525	5.62	5.44	-0.18
1529	5.37	5.17	-0.20
1623	5.84	6.35	+0.51
1624	7.34	8.17	+0.83
1625	5.44	6.00	+0.56
1675	4.52	4.76	+0.24
1676	4.92	5.60	+0.68
1712	8.17	8.19	+0.02
1713	5.19	5.54	+0.35
1714	6.47	6.57	+0.10
1754	2.43	2.38	-0.05
1755	2.49	2.43	-0.06
1757	3.26	3.57	+0.31
1758	7.16	6.94	-0.22
1762	2.73	2.65	-0.08
2084	3.49	3.17	-0.32
Average	4.91	5.05	+0.14

weaker acids. The average fat removed by the strong acid was 4.91 per cent, and by the weak acid it was 5.05 per cent. Thus an average of 0.14 per cent more fat was removed by the weaker acid. This weaker acid seemed to facilitate the fat determinations since emulsions were less likely to form and the extraneous materials settled below the side arm of the Röhrig tube better than with the stronger acid. It would be advisable to investigate further the effect of the concentration of the hydrochloric acid used in the acid hydrolysis method.

#### CONCLUSIONS

In this collaborative study of the acid hydrolysis method for fats in dog feeds too few collaborators participated for the results to be of much significance. All collaborators obtained more fat on all samples by the acid hydrolysis method than by the Bailey-Walker method with one ex-

ception, Sample 3. However, in a few cases there were wide variations in the results obtained by both methods. Since an acid hydrolysis of the residue after direct ether extraction gives considerable amounts of fat, further investigation should be made of the fatty materials removed by both methods. Slight modifications of the acid hydrolysis method possibly will prove advantageous.

It is recommended that this problem be further investigated and that collaborative work be continued.

## REPORT ON SELENIUM

By A. K. KLEIN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Although the collaborative results on selenium samples obtained in 1942 with the modified tentative method recommended by the Associate Referee in his 1941 report (*This Journal*, 24, 363) were generally satisfactory, certain observations of the collaborators made necessary an amplification of the published procedure. The resulting revision, outlined in the 1942 report (*Ibid.*, 25, 403), was submitted for further collaborative study in 1943.

Collaborators were supplied with a selenious acid solution of known selenium content in order to familiarize themselves with the method and also to enable them to standardize several of the reagents needed. In addition, they were given a quantity of dried, ground grapefruit rind known to be free of selenium and a solution marked "Unknown." The latter solution contained selenious acid equivalent to .8 micrograms of

TABLE 1.—*Collaborative results*

ANALYST	Se FOUND p.p.m.	AVERAGE p.p.m.	ANALYST	Se FOUND p.p.m.	AVERAGE p.p.m.
(A)	.76	.76	(E)	.81	.80
	.76			.73	
				.76	
(B)	.72	.73	(F)	.60	.67
	.73			.68	
				.72	
(C)	.80	.79	(G)	.90	.94
	.79			.98	
	.77				
(D)	.80	.76	Average		.78
	.81				
	.74				
	.74				
	.76				
	.77				

selenium per ml. in approximately 20 per cent nitric acid by volume. The collaborators were requested to add 10 ml. of the unknown to 10 grams of the fruit rind, to proceed with the analysis as revised in the 1942 report, and to report their results in parts per million of selenium. The correct value, necessarily, in this instance is .8 p.p.m. Table 1 summarizes the results of the collaborators.

#### COLLABORATORS

- (A) B. B. Westfall, U. S. Public Health Service, National Institute of Health, Bethesda, Md.
- (B) O. E. Olsen, South Dakota State College, Brookings, S. Dak.
- (C) D. W. Williams, Food and Drug Administration, San Francisco, Calif.
- (D) I. Schurman, Food and Drug Administration, Cincinnati, Ohio.
- (E) M. Beroza, Food and Drug Administration, Washington, D. C.
- (F) G. E. Keppel, Food and Drug Administration, Minneapolis, Minn.
- (G) C. C. Cooley, Food and Drug Administration, Seattle, Wash.

#### DISCUSSION

The collaborative results are obviously very good, although there is a tendency, except in one instance, to slightly low values. This, however, seems to be common to most micro methods involving several isolation steps wherein the final evaluation of the desired constituent is strictly quantitative and therefore not compensatory. One collaborator seemed slightly confused by the wording of the method, and this seeming ambiguity is corrected in the revised procedure presented in this report. Although the selenium content of the 1943 sample was less by 20 per cent than that of the 1942 sample, which contained 1 p.p.m. of the added element, the following mathematical interpretation indicates the superiority of the 1943 results over those of 1942.

Analysis of variance was applied to the two sets of collaborative results. By this means two types of errors of each method can be separated: (1) The variation of a single analyst in checking himself (here labeled "within analysts") and (2) the variation of any analyst in checking any other result (labeled "total"). In Table 2 the two sets of data are compared with regard to both of these types of errors (standard deviation). The derivation of the errors is also included for those interested in statistics.

The over-all standard deviation of the 1943 values is just half that of the values of 1942, and also the same ratio maintains for the standard deviation within analysts, namely .035 in 1943 versus .077 in 1942. This means that two times out of three the average analyst comes within .035 p.p.m. of his true average value, i.e. checks himself within about 4.5 per cent and 19 times in 20 he checks himself within .070 or about 9 per cent. The "total" or "over-all" error is about twice as great. There is a significant difference between analysts on both sets of data. Therefore the Associate Referee considers that he is justified in offering the following



TABLE 2.—Analyses of variance of the 1942 and 1943 collaborative results

SOURCE OF VARIATION	1942 DATA				1943 DATA			
	D/F*	SUM OF SQUARES OF DEVIATIONS FROM AVERAGES	MEAN SQUARES (VARIANCE)	STD. DEV. (ROOT MEAN SQUARES)	D/F*	SUM OF SQUARES OF DEVIATIONS FROM AVERAGES	MEAN SQUARES (VARIANCE)	STD. DEV. (ROOT MEAN SQUARES)
Total	23	.4709	.0205	.143	22	.1188	.0054	.073
Between Analysts	9	.3884	.0432		6	.0996	.0166	
Within Analysts	14	.0825	.0059	.077	16	.0192	.0012	.035

\* D/F indicates "degrees of freedom."

These data were compiled and interpreted by L. F. Knudsen, Food and Drug Administration, Washington, D. C.

i  
procedure as an official, first action, method for the determination of selenium especially in the range of 1–25 micrograms. Repeated experiments have demonstrated that it is equally well adapted to the estimation of much larger quantities (*loc. cit.*)

## SELENIUM

1

## REAGENTS

(a) *Sulfuric-nitric acid solution*.—To 50 ml. of  $\text{H}_2\text{SO}_4$  add 100 ml. of  $\text{HNO}_3$ . Cool the mixture before using.

(b) *Mercuric oxide fixative*.—Dissolve the oxide in  $\text{HNO}_3$  in the proportion of 5 grams to 100 ml. of the acid.

(c) *Hydrobromic acid-bromine solution*.—Concentrated. Mix 10 ml. of liquid Br with 990 ml. of conc. HBr. (The concentrated reagent grade of acid is offered commercially in two strengths: the constant boiling mixture or 48% (8.1 N) and the 40% strength (7 N).)

(d) *Hydrobromic acid—bromine solution*.—Dilute. To 5 ml. of conc. HBr add 10 ml. of saturated Br water and dilute to 100 ml. with water.

(e) *Sulfur dioxide*.—The gas supplied in commercial cylinders is free of Se.

(f) *Hydroxylamine hydrochloride solution*.—10% W/V in water.

(g) *Phenol solution*.—5% W/V in water.

(h) *Standard sodium thiosulfate solution*.—Prepare from accurately standardized 0.1 N reagent with recently boiled water. Before adjusting to final volume add 5 ml. of amyl alcohol per liter and shake vigorously. 1 ml. of .001 N thiosulfate is theoretically equivalent to 19.8 micrograms of Se. (For the estimation of Se in quantities greater than 50–75 micrograms proportionately stronger concentrations of thiosulfate are required.)

(i) *Standard iodine solution*.—Prepare from 0.1 N reagent. Before final dilution add KI in the proportion of 20 grams/liter. Dilute to like normality of thiosulfate.

(j) *Standard selenium solution*.—Dissolve 250 mg. of C.P. selenium in conc. HBr-Br. solution (1 ml. liquid Br + 25 ml. conc. HBr, both of which have been distilled). After complete solution has been effected, *almost* neutralize the excess Br with the  $\text{SO}_2$  gas while shaking vigorously. Complete the neutralization by adding the phenol solution dropwise in slight excess. Make to 250 ml. with water. ( $\text{SO}_2$  must not be present in excess because it would then reduce the selenious acid to the element.) If too much  $\text{SO}_2$  has been used, add Br water until the color of the selenite

solution is slightly but definitely yellow and then complete the neutralization with the phenol. (Unless the analyst is positive that the Se reagent is pure, he must purify it. To do this dissolve ca. a gram of the Se in an excess of the conc. HBr-Br, precipitate with  $\text{SO}_2$ , warm on the steam bath for 30 minutes, cool, filter first wash free of acids with water and then wash with small portions of ethyl alcohol, dry at  $100^\circ\text{C}$ . for 1 hour, and prepare the 1 mg./ml. standard as described above. This precaution is necessary since the Se solution serves as the ultimate standard in the determination.) Make appropriate dilutions of the concentrated reagent by adding water, and do not allow the acidity, determined by titration, to fall below .05  $N$ , since neutral or very slightly acid solutions of dilute selenious acid tend to oxidize and lose their titer. A dilution of 20 microgram of Se/ml. is convenient for micro determinations, for then it is almost chemically equivalent to accurately prepared .001  $N$  thiosulfate (1 ml. of .001  $N$  thiosulfate is equivalent to 19.8 micrograms of Se).

(k) *Soluble starch indicator.*—0.5% W/V.

## 2

## APPARATUS

An all-glass distillation outfit consisting of 250 ml. round-bottomed flask, still head, thermometer registering to  $135^\circ\text{C}$ ., and condenser with dipping end.

## 3

## DETERMINATION

Place 5–10 grams (dry weight) of the sample in a 600–800 ml. Pyrex beaker and add 10 ml. of the  $\text{HgO}$  fixative followed by 150 ml. of the  $\text{H}_2\text{SO}_4\text{-HNO}_3$ . Mix thoroly at once and place on the steam bath for 30 minutes, stirring intermittently. If the product is rich in Se, use 1 gram of representative material. To dry leafy products such as hop leaves, which oxidize violently, add 25 ml. of water before applying the fixative. Heat over the burner (not full flame) until the digestion mass lightens and then turns brown. Remove the flame, cool, and after adding 10 ml. of concentrated  $\text{HNO}_3$ , again heat until the first brown appears. Repeat this operation at least twice and then heat until the liquid turns a distinct brown (not black) or until  $\text{SO}_2$  fumes appear. (It is imperative that the excess  $\text{HNO}_3$  be expelled and that the organic matter be sufficiently oxidized so that the Br reagent subsequently added is not reduced, but prolonged fuming to  $\text{SO}_2$  is to be avoided.) As such products as molasses and honey, principally sugars, react vigorously with  $\text{HNO}_3$  remove such samples from the steam bath until the reaction subsides and then proceed in the usual manner.

Cool the digest and transfer with two 25 ml. portions of water to the distilling flask. (If the digestion has been carried out in the 250 ml. distilling flask, it is necessary even then to add 50 ml. of water so that the HBr will distil subsequently as the liquid and not the vapor.) Rinse the beaker carefully with 25 ml. of the HBr-Br and add to the cooled digest and washings. (If the constant-boiling grade of HBr has not been used, an equivalent volume of a less concentrated reagent must be added, e.g., 30 ml. of 40% strength and the distillate must have an acidity of ca. 2.5  $N$ .) After swirling the flask, distil until the temperature of distillation reaches  $130^\circ\text{C}$ . into a 125 ml. Erlenmeyer flask, marked at 50, 75, and 100 ml., containing 5 ml. of the conc. HBr and surrounded by cold water. During the distillation lift the tip of the condenser out of the liquid in the flask after all the Br and ca. 15 ml. of the acid have distilled. (Free Br should distil in the beginning, indicating an excess of the reagent. If this is not the case, stop the distillation, cool, and add an additional 10 ml. of the HBr-Br reagent. This contingency arises only with insufficient digestion of sample.) Rinse the condenser tip carefully with two portions of no more than 2 ml. each of water. Between analyses rinse the condenser tube free of fatty and

waxy material with hot water but *do not* add the rinsings to the distillate. For the next three steps it is assumed that the distillate contains no fats, waxes, or other insoluble matter. (1) If the volume of the distillate and rinsings is 75 ml. or less, pass in  $\text{SO}_2$  in excess (about a half-minute after complete decolorization of the bromine), add 1 ml. of the  $\text{NH}_4\text{OH-HCl}$ , and place mixture on an active steam bath for 30 minutes. Cap the flasks with watch-glasses during the various heat treatments. (2) If the volume of the distillate is between 75 and 100 ml., reduce with  $\text{SO}_2$  and the  $\text{NH}_4\text{OH-HCl}$  as directed in (1), add several glass beads, bring just to incipient boiling, and complete the reduction at once with the 30-minute steam-bath treatment. (3) If the volume of the distillate should exceed 100 ml., transfer to a 200 ml. Erlenmeyer flask and complete the transfer with four successive 2 ml. rinsings with water, delivered from a pipet, and combine with original distillate. Add 10 ml. of the concentrated  $\text{HBr}$ , reduce with the  $\text{SO}_2$  and  $\text{NH}_4\text{OH-HCl}$  in the usual manner, add several glass beads, bring to incipient boiling, and place the flasks at once on the steam bath for 30 minutes. (When the volume of the solution exceeds 100 ml., the recovery of Se may be slightly low.)

If the distillate contains fats, waxes, or other insoluble material, filter off with suction on asbestos and rinse receiver flask carefully with four 2 ml. portions of water from a pipet. Use the successive washings in turn to rinse the asbestos filter. Transfer the combined filtrate to a 125 ml. Erlenmeyer flask (200 ml. flask if the volume of filtrate exceeds 100 ml.), and complete the transfer with four 2 ml. portions of water also delivered from a pipet. According to the final volume of solution, whether less than 75 ml., between 75 and 100 ml., or in excess of 100 ml., proceed with the addition of the reagents and the heat treatment exactly as directed previously. (Se is reduced rapidly from an acid solution 2.5 *N* or more; steps 2 and 3 are necessary because the acidity is less than 2.5 *N* in these instances.)

For the assay of products of high Se content (more than 1,000 micrograms in the amount of material analyzed) use 50 ml. of the  $\text{HBr-Br}$  (or its equivalent volume of a less concentrated grade) for the initial distillation. To such samples add 75 ml. of water during the rinsing instead of the usual 50 ml. After the usual distillation, disconnect the apparatus and rinse the condenser tube with 5 ml. of water, which is added directly to the distillate. Heat the residue in the distillation flask to incipient fumes of  $\text{SO}_3$ , cool, add 5 ml. of  $\text{HClO}_4$ , and heat to fuming. Repeat the  $\text{HClO}_4$  oxidation. (This treatment is necessary for substances like vetches and seedlings, which contain particularly refractory Se compounds.) Cool the digest, add two 25 ml. portions of water and then 25 ml. of the  $\text{HBr-Br}$ , and distil to  $130^\circ\text{C}$ . in the usual manner. Combine all distillates and if fats, waxes, or other insoluble matter are present, filter, and wash as previously directed. In either case, adjust to exactly 250 ml. in a volumetric flask with water, pipet 75 ml. into a 125 ml. Erlenmeyer flask, reduce the Se with excess  $\text{SO}_2$  and  $\text{NH}_4\text{OH-HCl}$ , and complete the reduction by heating on the steam bath for 30 minutes.

Place the flasks in cold water (ca.  $20^\circ\text{C}$ .) for a like period and then with suction collect the Se on an asbestos pad contained in the filtration vessel, Figure 1. Rinse the precipitation flask and pad with 5 successive 1 ml. portions of water from a pipet, and then hold the mouth of the flask before an air vent to remove the last traces of  $\text{SO}_2$ .

Insert the filtration vessel into the titrating tube and dissolve the Se with 1 ml. of the dilute  $\text{HBr-Br}$ , first adding the reagent from a pipet to the flask and then transferring carefully to the pad. When the Se has dissolved, apply gentle suction and repeat the operation with an additional 1 ml. of the dilute  $\text{HBr-Br}$ . Finally rinse flask and pad with 3 successive 1 ml. portions of water, collecting the filtrate

before each addition. 2 ml. of the dilute HBr-Br reagent is sufficient for Se up to 500 micrograms. When more is present, use proportionately more reagent and rinse water.

Agitate the filtrate with pipet stirrer and dispel excess Br with 3 drops of the phenol reagent. Using the stirrer as a pipet, rinse the walls of the vessel several times with the solution to neutralize every trace of Br. Immerse the titrating tube up to two-thirds of its length in hot water for 5 minutes, stirring intermittently. (Heating is required to complete the reaction between the Br and phenol.) Then place the

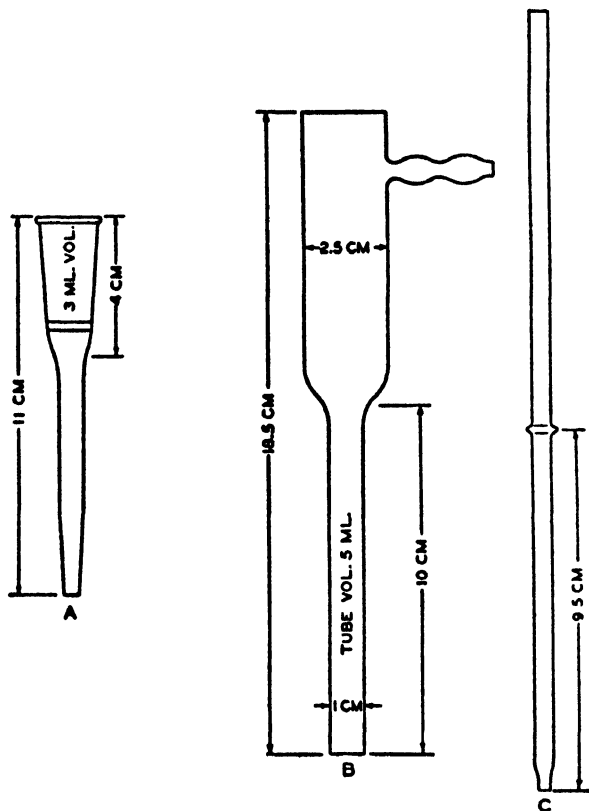


FIG. 1.—Filtration and titration tubes and pipet.

vessel in cold water for at least 5 minutes. (The Norris-Fay titration<sup>1</sup> works best when the solution is below 25°C.)

Using the original precipitate of Se as a guide, add at least a 50% excess of the appropriate strength of the standard thiosulfate reagent and 3 drops of the starch indicator. After stirring, add the standard I until a permanent blue color appears. If less than 1 ml. of I is required, add sufficient thiosulfate so that at least 1 ml. of I is required. Then titrate to a colorless end point with thiosulfate, adding the reagent in increments of .01 ml. as the end point is approached.

<sup>1</sup> *Am. Chem. J.*, 18, 705 (1896).

## 4

## CALCULATION AND STANDARDIZATION

## (Cross Titration)

To 2 ml. of dilute HBr (5+95) contained in the titrating tube add 3 ml. of water and 3 ml. of standard I. (The HBr must have been previously distilled.) Titrate with the standard thiosulfate and toward the end add 3 drops of the starch indicator. Complete the titration as directed in the sample determination and obtain the thiosulfate equivalent of the iodine.

## 5

## STANDARDIZATION OF THIOSULFATE

Add 2 ml. of the dilute HBr (5+95) to an appropriate volume of standard Se solution and after the addition of 50% excess thiosulfate continue the titration in exactly the manner described in 4. Obtain the Se equivalent of the thiosulfate. (No matter how carefully prepared, dilute solutions of both thiosulfate and iodine slowly deteriorate. They must, therefore, be standardized frequently.)

## 6

## SAMPLE CALCULATION

Multiply the net thiosulfate sample titer in ml. by the Se equivalent to obtain the quantity of Se in the sample.

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REPORT ON SALT IN EGG PRODUCTS

By L. C. MITCHELL, *Associate Referee*, and WILLIAM HORWITZ  
(U. S. Food and Drug Administration, Minneapolis, Minn.)

The following instructions were furnished the collaborators relative to the application of the open Carius method for the determination of chlorine and/or added salt in egg products.

## DETERMINATION

(a) From the well-mixed sample, 1(a), (b), (c), or (d), weigh accurately, by difference, ca. 4 grams of yolks, 7 grams of whole eggs, or 10 grams of whites, or transfer 2 grams of dried whole eggs or yolks, or 1 gram of dried whites into a 300 ml. Erlenmeyer flask; add known volume of 0.1 N  $\text{AgNO}_3$  in slight excess and 20 ml. of  $\text{HNO}_3$ , and place mixture on steam bath for 15-30 minutes. Add 15 ml. of 5%  $\text{KMnO}_4$  solution and allow mixture to stand 60-90 minutes longer on steam bath. Cool to 25° or less, add 75 ml. of water, 1 ml. of nitrobenzene (or 1 ml. of nitrobenzene for each 50 mg. of salt present), stopper flask, and shake vigorously to coagulate the precipitate. Add 5 ml. of saturated ferric alum indicator and titrate with 0.1 N thiocyanate solution to an end point that persists after the solution has stood 15 minutes. From the number of ml. of  $\text{AgNO}_3$  used, calculate quantity of NaCl, after deducting blank run on reagents, using ca. 0.25 gram of sucrose instead of egg.

(b) Repeat the determination after addition of 1 and 10% NaCl.

(c) Repeat the above work including blank, using the official method, *Methods of Analysis*, A.O.A.C., 1940, 311, 15, specifying the alternative method used.

Please report the percentage of salt by both the official and open Carius methods, together with the percentage recovery of the added salt by both methods.

TABLE 1.—*Collaborative results on determination of Cl, calculated as NaCl, by official (1) and rapid (2) methods*

COLLABORATOR	TYPE EGG SAMPLE	NaCl		RECOVERY OF ADDED SALT			
		(1)	(2)	1% ADDED		10% ADDED	
		per cent	per cent	per cent	per cent	per cent	per cent
1	Dried whole	1.02	1.01	98.4	96.8	99.6	99.3
	Liquid fresh whole	0.26	0.27	100.4	97.8	100.1	100.0
2	Dried whole	1.02	1.02	98.0	98.0	100.0	100.0
		1.02	1.02	98.5	97.0	99.5	100.0
	Frozen whole	0.31	0.30	98.5	98.0	99.0	100.4
		0.31	0.31	98.5	100.5	98.7	100.3
3	Dried whole	1.11	1.09	98.6	96.3	100.9	99.8
		1.11	1.08	100.0	96.3	101.4	100.0
	Liquid	0.28	0.25	97.2	95.2	100.5	101.1
		0.29	0.26	98.8	95.7	100.6	101.3
				98.1			
4	Dried whole	1.02	1.02	100.6	95.3	99.4	98.2
		1.05	1.03	98.8	91.3	100.6	99.4
		1.05	1.03	98.5	91.3		99.5
	Frozen whole	0.28	0.28	101.0	84.5	101.2	103.5
		0.28	0.27	100.0	90.5	103.1	100.7
			0.28	101.5			
5	Dried whole	1.04	0.96	97.5	99.2	99.8	98.2
		1.03	0.96	96.9	100.6	99.6	98.0
6	Com. salted yolk	7.51	7.53				
		7.51	7.56				
		7.52	7.57				
7	Dried whole	1.01	1.00	99.8	100.2	100.0	99.9
		1.01	1.00	99.3	98.8	99.9	99.8
		1.01	1.00	100.2	100.2	100.0	99.8
8	Dried whole	1.02	1.02	102.5	97.5	100.6	100.0
9	Dried whole	1.06	0.97	101	98	100.7	100.3
		1.06	0.97	101	96	100.7	100.1
	Dried Yolk	1.63	1.50	93	97	100.2	100.4
		1.63	1.52	95	100	100.2	100.7
			1.40	97			
			1.41	96			
	Liquid yolks	0.29	0.24			100.1 <sup>a</sup>	100.1 <sup>a</sup>
		0.29	0.30			100.1 <sup>a</sup>	100.1 <sup>a</sup>
	Liquid whites	0.27	0.29	99.5 <sup>b</sup>	99.0 <sup>b</sup>		
		0.30	0.28	99.5 <sup>b</sup>	99.5 <sup>b</sup>		

<sup>a</sup> Added salt 5% instead of 10%.<sup>b</sup> Added salt 2% instead of 1%.

## NOTES

1. Titrations should be made at temperatures of 25° or below, as is customary in other titrations with thiocyanate. The solution is yellow green before the end point and a yellow orange at the end point. At the first permanent color change, note the buret reading and time, stopper flask, shake vigorously, and allow to stand 15 minutes. At the end of this time, if the solution has faded, add the thiocyanate solu-

tion in half-drop portions to the end point color. Add additional amount of solution used to the original reading to obtain the total titration. (See *Ind. Eng. Chem., Anal. Ed.*, 7, 38 (1935).

2. The salt may be conveniently added in the form of a solution containing 2 grams per liter, standardized against the standard  $\text{AgNO}_3$  solution. 10 ml. and 100 ml. added to 2 grams of dried eggs will give 1 and 10%, respectively, of added salt. The measured amount of salt solution can be evaporated almost to dryness on the steam bath before proceeding by the two methods. 10 ml. of 0.1  $N$   $\text{AgNO}_3$  is sufficient for 2 grams of dried whole eggs, or this amount of dried eggs with 1% added salt, and 50 ml. is sufficient with 10% added salt.

Known samples were not submitted to the collaborators, but they were asked to do the work on both liquid (fresh or frozen) and dried eggs.

The Associate Referee appreciates the generous cooperation of the following collaborators, all members of the Food and Drug Administration: F. B. Jones, New York; F. J. McNall, Cincinnati; A. G. Buell, San Francisco; H. O. Fallscheer, Seattle; G. M. Johnson, St. Louis; G. E. Keppel, S. H. Perlmutter, Wm. Horwitz, Minneapolis; E. O. Haenni and Elizabeth J. Billett, Washington.

The collaborative results are given in Table 1.

#### COMMENTS OF COLLABORATORS

*F. B. Jones.*—Alternative method (1) was used in the official method.

*F. J. McNall.*—Alternative method (1) was used in the official method.

*A. G. Buell.*—Alternative method (2) was used in the official method.

*H. O. Fallscheer.*—Alternative method (1) was used in the official method. No difficulty was experienced in using either method.

*G. M. Johnson.*—Although there is some difference in the recovery by the two methods, I feel that the open Carius is equivalent to the present official method. However, I would suggest that about 30 ml. of 5%  $\text{KMnO}_4$  be used instead of 15 ml. as this gives a less colored solution. Personally I do not like the use of the nitrobenzene. I would prefer filtering before titrating the excess silver.

*E. C. Haenni.*—Although consistent with each other, the results by the open Carius method on the dried eggs tend to be lower. However, it appears to be satisfactory for all practical purposes.

Apparently with small amounts of chloride, the rapid method gives somewhat lower results, and neither the official nor the rapid method completely recovers an amount of added salt approximately equal to that originally present (1 per cent for dried whole eggs). However, the recovery of large quantities of salt either as added salt (10 per cent for dried whole eggs) or in a commercial product (Collaborator 6) is very satisfactory.

#### REPORT ON SALICYLIC ACID IN HAIR LOTIONS

By HENRY R. BOND (U. S. Food & Drug Administration,  
Kansas City, Mo.), Associate Referee

The method of assay for salicylic acid in hair lotions recommended for tentative adoption in 1941 represented a revision of the method originally

sent to collaborators for their guidance. The revision was based upon critical comment made by the collaborators.

In order to present for consideration further results obtained through actual use of the revised method of assay, the Associate Referee forwarded to collaborators another sample of similar composition to the one outlined in his 1941 report. However, in the current sample, resorcinol monoacetate and chloral hydrate were not included as components.

#### METHOD

Acidify 25 ml. of sample in a 250 ml. beaker with 2 ml. of 10% HCl. Dealcoholize by heating at not more than 70°C., if possible by use of a current of air at room temperature. Transfer to a separatory funnel and dilute with water to ca. 25 ml. Since  $\text{CHCl}_3$  is to be used later as the extracting solvent, make the transfer by washing from beaker to separator with 2 or 3 portions of  $\text{CHCl}_3$ , totaling 25 ml., repeating the washing with water in portions totaling 25 ml., thus obtaining the indicated aqueous dilution.

Extract with four 25 ml. portions (includes the 25 ml. used in transfer of sample to separator). Wash each  $\text{CHCl}_3$  extract with 5 ml. of water and filter into a 150 ml. beaker through a  $\text{CHCl}_3$ -saturated pledget of cotton. Wash the 5 ml. of water with  $\text{CHCl}_3$ , filtering these washings into the same beaker. Evaporate the  $\text{CHCl}_3$  on a steam bath to a volume of 20–25 ml., allowing this amount to evaporate spontaneously to a volume of 5 ml.

Transfer the remaining 5 ml. to a separatory funnel, with enough  $\text{CHCl}_3$  as a rinsing agent to make a volume of ca. 30 ml. in the separator. Extract with three 5 ml. portions of 5% aqueous  $\text{NaHCO}_3$  solution and one 5 ml. portion of water. Wash the combined extracts with a 10 ml. portion of  $\text{CHCl}_3$  and discard the  $\text{CHCl}_3$ .

Filter the combined aqueous bicarbonate solutions through filter paper into a 100 ml. volumetric flask. Rinse the separators and wash the filter with water until the volume of filtrate reaches the 100 ml. mark. Agitate the flask to obtain uniformity of solution.

Proceed as directed in *Methods of Analysis*, A.O.A.C., 1940, 568, 27, beginning, "Transfer aliquot of this solution." Before adding 0.1 N bromide-bromate solution, carefully neutralize the aliquot in the iodine flask to liberate  $\text{CO}_2$  from the bicarbonate present, then make alkaline with one drop of 10% NaOH solution and continue as directed. 1 ml. of 0.1 N bromine solution = 0.0023 gram of salicylic acid.

#### RESULTS

Collaborators reported the following percentages of salicylic acid in the sample, indicated in grams per 100 ml.:

COLLABORATOR*	SALICYLIC ACID gram/100 ml.
R. Hyatt, Cincinnati, Ohio	0.990; 0.990; 0.980
F. M. Garfield, St. Louis, Mo.	0.970; 0.970
G. E. Keppel, Minneapolis, Minn.	0.990; 0.990; 0.990
S. H. Perlmutter, Minneapolis, Minn.	0.990; 0.980; 0.990; 0.980
E. H. Berry, Chicago, Ill.	0.968; 0.964; 0.979
Associate Referee	0.979; 0.975; 0.969; 0.970
Average	0.980
Theoretical	0.997
Per cent Recovery	98.30

\* All members of the Food and Drug Administration.



## COMMENTS

Comments on the procedure were made by Berry and Garfield, who experienced difficulty through the formation of emulsions in the extraction with the sodium bicarbonate. The Associate Referee also experienced some difficulty at the same point, but concluded that more careful agitation of the mixture would rectify the trouble.

## DISCUSSION

The method of assay, in view of the collaborators' results, appears to be satisfactory. Difficulties encountered by collaborators in 1941 have been overcome through use of the revised method.

## CONTRIBUTED PAPERS

### STEAM DISTILLATION—A NEW PROCEDURE FOR THE DETERMINATION OF CARBONATE CO<sub>2</sub>

By W. M. SHAW and W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The present investigation was undertaken primarily to develop a precise and expeditious procedure for the determination of minute incidences of carbonates in soils. The need for such a procedure had developed in the course of certain studies as to the reactive capacities of soils, differential dissolutions of limestone and dolomite, and the carbonatation of calcium silicates.

Since the proposed procedure called for novel apparatus and new technic, it was necessary to make a detailed investigation of the several steps. In the early part of this study calcic and dolomitic limestones and magnesite of variant fineness were used. After technic and apparatus for decomposition and CO<sub>2</sub> absorption had been developed, titration of the absorbed CO<sub>2</sub> remained the limiting factor of precision. Further survey of the literature and additional laboratory studies were directed to this factor, and finally the procedure was adapted to the determination of carbonates in soils. The present contribution, therefore, is divided into four parts:

- I. Development of apparatus and technic
- II. Investigation of titrimetric methods
- III. Procedure for inorganic materials
- IV. Adaptation for soil carbonates

#### *I. DEVELOPMENT OF APPARATUS AND TECHNIC*

The unique feature of the proposed procedure is the use of steam to heat and agitate the analytical system and to sweep the liberated CO<sub>2</sub> into the absorption chamber. Chief advantages are speed and precision. The dissolution of the most recalcitrant limestone is accomplished in two minutes, the sweeping of the CO<sub>2</sub> in one minute, and the over-all time of apparatus tie-up per sample is five minutes.

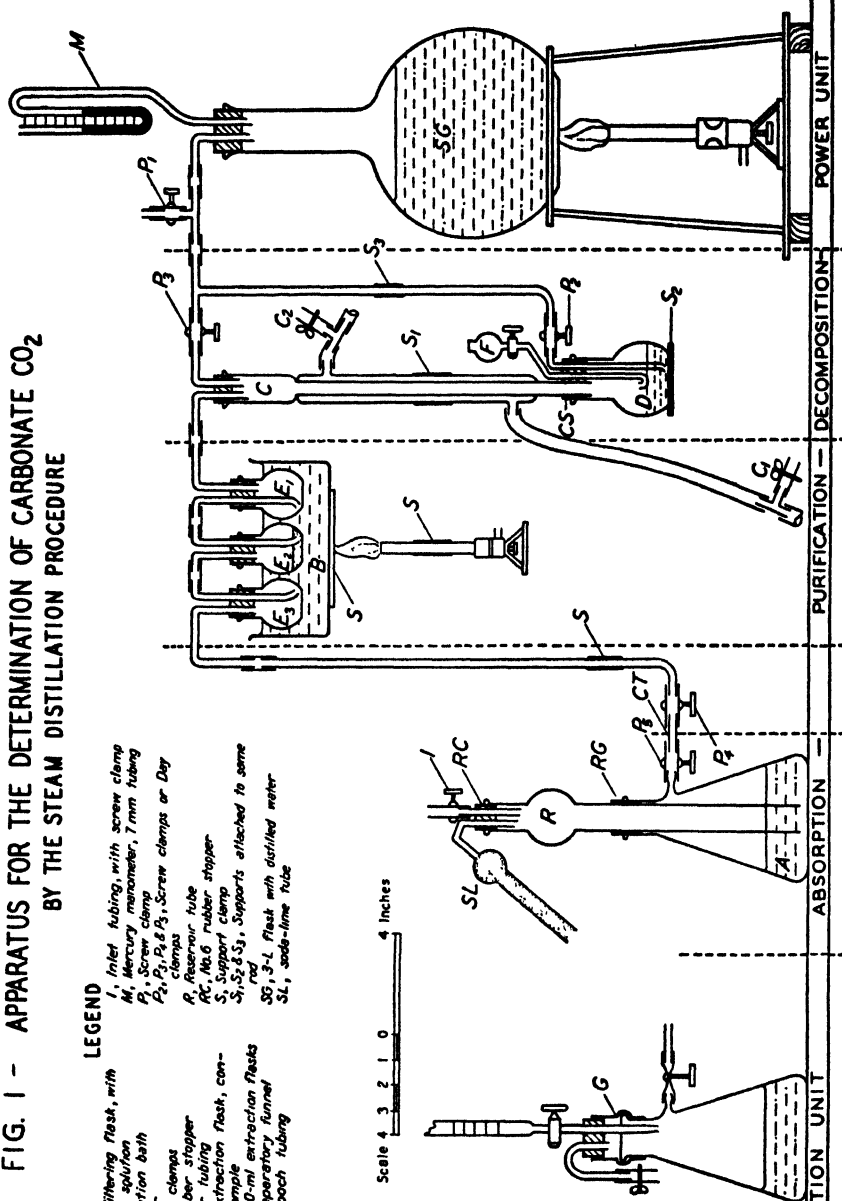
The utilization of steam is facilitated by the coordination of the size and shape of the several parts of the apparatus, especially the absorption unit. This consists of a 500 ml. suction flask provided with a special reservoir tube that can be moved perpendicularly inside the flask while maintaining an airtight joint. As the liberated CO<sub>2</sub> passes into the absorption flask, the liquid is forced up into the reservoir tube. It is therefore necessary to provide a sufficient volume of the absorbent to maintain a seal with the tube. It is essential that the capacity of the tube be adequate to accommodate displacement of absorbent. These volume relationships are

FIG. 1 - APPARATUS FOR THE DETERMINATION OF CARBONATE CO<sub>2</sub>  
BY THE STEAM DISTILLATION PROCEDURE

LEGEND

- A, 500-ml. filtering flask, with absorbent solution  
B, CaCl<sub>2</sub> solution bath  
C, Condenser  
C<sub>1</sub>, Condenser  
C<sub>2</sub>, Pinch clamps  
C<sub>3</sub>, No. 7 rubber stopper  
C<sub>4</sub>, Connector tubing  
C<sub>5</sub>, 150 ml. extraction flask, containing sample  
E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, 50-ml. extraction flasks  
F, 30-ml. separator funnel  
G, 2 inch Gooch tube  
I, Inlet tubing, with screw clamp  
M, Mercury manometer, 7 mm tubing  
P<sub>1</sub>, Screw clamp  
P<sub>2</sub>, P<sub>3</sub>, P<sub>4</sub>, P<sub>5</sub>, Screw clamps or Day clamps  
R, Reservoir tube  
RC, No. 6 rubber stopper  
S, Support clamp  
S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, Supports attached to same rod  
SG, 3-L. flask with distilled water  
SL, soda-lime tube

Scale 4 3 2 1 0 4 Inches



governed by the amount of the liberated  $\text{CO}_2$ , i.e., carbonate charge, and by the over-all volume of air present in the apparatus at the beginning of the determination. This latter factor dictated that the decomposition flask should be small. The lateral enlargement of the upright tube in the absorption chamber affords increased capacity without undue height and serves to minimize back pressure during the final stages of the determination.

When the absorption unit is closed completely, the reservoir tube is slid upward until its lower end is just inside the absorption chamber and the trapped  $\text{CO}_2$  is absorbed by vigorous agitation of the absorbent for 30 seconds. The other accessories that facilitate the use of steam are shown in the accompanying diagram, Fig. 1.

#### FACTORS THAT AFFECT ACCURACY OF PROPOSED PROCEDURE

In an effort to establish flexibility of the assigned timing and other experimental conditions, pure carbonates, limestones, and dolomites were used and the following factors were considered: (a) Volatility of the dissolvent acid during steam distillation; (b) time required for decomposition of carbonates; (c) time required for the "sweeping" of  $\text{CO}_2$ ; (d) elimination of gaseous impurities; (e) choice of  $\text{CO}_2$  absorbent; (f) effectiveness of barium hydroxide and sodium hydroxide absorbent solutions as affected by  $\text{CO}_2$ -load and period of agitation.

#### DILUTE PERCHLORIC ACID VS. DILUTE HYDROCHLORIC ACID AS DISSOLVENTS

Dilute hydrochloric acid is usually employed in the analytical decomposition of carbonates, but it was anticipated that the volatility of this acid might become a problem in rapid distillations with steam. The next choice was perchloric, since this acid is nonvolatile in dilute solution and because of the solubility of its salts. When 70 per cent perchloric acid is diluted 1+9, the concentration of the resultant solution is about "normal." Five ml. of this diluted acid will dissolve a 0.25 gram sample of calcite, the maximal charge recommended, and 25 ml. affords a 400 per cent excess. Since the analytical charge is washed down with 40 ml. of water, the dissolvent will be approximately  $\frac{1}{3}$  normal during the steam digestion.

The volatility of the perchloric acid was compared with that of hydrochloric acid by titrations of the distillates obtained by 5 minute passages of steam through the dilute acids. The condensate from the perchloric acid distillation was alkaline to methyl orange, as was the control distillate from distilled water, whereas the condensate from the hydrochloric acid distillation required 0.5 ml. of 0.1 *N* alkali for neutralization. Similarly, the distillation from dilute perchloric acid containing 5 per cent of stannous chloride developed an acidity of 0.1 ml. of 0.1 *N* acid. When a scrubber bulb containing water was interposed between the reaction flask and the condenser, the condensate from the dilute hydrochloric acid-

steam passage was neutral. Hence, when the current of steam is to pass through an aqueous scrubber, dilute hydrochloric acid is as satisfactory as perchloric acid of like dilution.

#### TIME NECESSARY FOR THE COMPLETE DISSOLUTION OF CARBONATES

The completeness of decomposition of pure carbonates can be recognized readily by the clarity of the resultant solution or by the absence of bubbles. This observation cannot be depended upon, however, when the decomposition mixture contains heavy residues or soil suspensions. In the use of the older procedures it has been necessary, therefore, to follow through the complete determination with variant periods of digestion and aspiration to establish requirements for the decomposition of the recalcitrant types of mineral carbonates. Such arbitrarily prescribed digestions as "30 minutes digestion with aspiration" do not assure reproducibility of the several factors—type, amount, and particle size of limestone, vigor of agitation, and rate of aspiration. These conditions are variants and the imposed period, therefore, may be of unnecessary duration to assure complete dissolution of the analytical charge and total expulsion of  $\text{CO}_2$ .

In the proposed procedure, however, the decomposition period is fixed as an adequacy for all carbonates, regardless of particle size. So long as an analytical charge of carbonate is undergoing dissolution, the liberated  $\text{CO}_2$  will continue to bubble through the liquid in the purification tube, or scrubber, beyond the reflux condenser. When bubbling ceases and the liquid begins to back up in the inlet tube, the carbonate decomposition is complete. Calcic limestones of 40-mesh or finer are dissolved in from 10 to 20 seconds after refluxing begins; dolomitic limestones of 40-mesh or finer dissolve within 1 minute after refluxing begins. A refluxing period of two minutes, therefore, assures twice the time necessary for the complete decomposition of the most resistant mineral carbonate. The completion of the carbonate decomposition can be recognized by either noting the pressure indicated by the bubbling tube or by the imposition of two minutes of refluxing.

#### PROVISION FOR GASEOUS IMPURITIES

The steam current carries acidic vapors from the decomposition acid and, in the case of sulfide-bearing carbonates, also hydrogen sulfide. Most acidic vapors are trapped readily by the interposition of an aqueous scrubber bulb, but a more complicated system of purifiers is required for the elimination of hydrogen sulfide. Two types of purifiers, therefore, are described.

(a) *The Simple Type—applicable in absence of hydrogen sulfide.*—The 50 ml. extraction flask, interposed between the condenser and the absorption unit, should contain about 10 ml. of water initially. In addition to inlet and outlet, the flask is provided with a syphon tube for either introduction or withdrawal of water. This flask is a precautionary device to

trap any acid spray or foam, and also serves to indicate progress of carbonate dissolution during the reflux digestion.

(b) *The Complete Type*—applicable to all cases and essential in the presence of sulfides.—Some limestones contain sulfides and it is imperative, therefore, to interpose an appropriate absorbent to remove the hydrogen sulfide liberated during dissolution of the analytical charge. When the apparatus is swept by a current of steam, certain mechanical difficulties may arise. Steam condensation results in the flooding of the absorption tubes, and the force of the steam current causes the ejection of the absorbent from the usual type of containers. These difficulties were overcome ultimately by the appropriate shaping of the absorbent bulbs and by temperature control. A 50 ml. extraction flask was found most suitable as the container for 20 ml. of the 5 per cent silver sulfate suspension in 1+19 sulfuric acid. A similar flask serves as a splash trap in the rear position and another serves as a back-pressure trap in the forward position. The connecting tubes are arranged so that the bulk of the liquid returns to the middle flask during the decomposition stage. The three flasks are held together by a common wooden clamp, and are kept partially submerged in a bath of an aqueous solution of calcium chloride (550 grams/liter of water) heated at 110° in a 170 mm. Pyrex crystallizing dish three-fourths filled.

#### QUANTITATIVE CO<sub>2</sub> ABSORPTION

Most of the older types of absorption tubes are patterned after the Truog (7) bead-filled tower, which stands in a 500 ml. suction flask and into which the gases must enter first. The A.O.A.C. method (2) calls for a direct-entry upright-type tube 25 inches high and filled with alternate pockets of glass beads and rods, as prescribed by MacIntire and Willis. From their study of various types of absorbers, Martin and Green (5) concluded: "Although it is generally assumed that a bead column is highly efficient in absorption, on account of the large surface of the absorbent exposed to the gases, these studies show it to be less efficient than either the spiral or the Milligan absorbers." Friedman and Kendall (4) found that the CO<sub>2</sub> absorption in the Truog type of absorber was incomplete with sodium hydroxide of concentrations less than half normal. Using the same type of apparatus, Adams (1) found 100 ml. of 0.5 *N* sodium hydroxide necessary for determinations, the theoretical requirements of which were only 10–30 ml. of the hydroxide.

Wells, May, and Senseman (8) proposed the substitution of a sinter-bottomed Bruce and Bent (3) tube for the bead-tower type of absorber. They added 0.5 per cent of butyl alcohol and obtained 100 per cent efficiency with 0.1 *N* sodium hydroxide up to 94 per cent of its theoretical capacity with an air flow of 100 ml. per minute. Thomas (6) used a similar type of tube and obtained absolute absorption with 0.0052 *N* sodium hydroxide that contained 0.4 per cent butyl alcohol in the passage of air

currents carrying 320 p.p.m. of  $\text{CO}_2$  at 330 ml. per minute. He concluded that for assurance of absolute absorption of liberated  $\text{CO}_2$ , the air flow should be rapid enough to cause the formation of a foam in the absorbent. In this laboratory the type of tube suggested by Wells, May, and Senseman (8) was found to be effective, and its use obviates the back titration of large excesses of alkali. The absorption apparatus to be described has been found to be 100 per cent effective and without restriction as to either concentration of absorbent or rate of air flow.

#### THE $\text{CO}_2$ ABSORPTION APPARATUS

The general description of the absorption apparatus given previously in this paper will now be supplemented with some functional details. The absorption unit resembles somewhat the apparatus suggested by Wells, May, and Senseman (8), but the two types of apparatus are widely dissimilar in construction of the absorption tube and manner of its utilization. The tube adopted is of Pyrex, 25 mm. in diameter and 330 ml. in length. It is open at both ends and has a capacity sufficient to accommodate the volume of absorbent displaced by the gases that enter the flask. The tube does not function as an absorption vessel, its primary function being to maintain a seal with the absorbent solution in the flask that receives the mixture of air and  $\text{CO}_2$  during the determination.

The size and shape of the tube are governed by several considerations. One of these is the initial volume of air in the apparatus. The volume of  $\text{CO}_2$ -free air in the 150 ml. decomposition flask amounts to 60–70 ml. after introduction of the analytical charge and hot water. To restrict the air volume and to preclude  $\text{CO}_2$  contamination, a rapid flow of steam is maintained downward through the condenser during the intervals between determinations. The second factor is the variation in the volume of  $\text{CO}_2$  liberated from the analytical charge. A 0.25 gram sample of calcium carbonate will yield 0.1100 gram, or 62 ml., of  $\text{CO}_2$  at  $30^\circ\text{C}$ . The volume of the resultant mixture of air and  $\text{CO}_2$  will be approximately 130 ml. To this must be added the influx of steam that enters the absorption unit during the last 30 seconds of the determination. From these computations the operating volume for the reservoir tube was fixed at 250 ml. As a safeguard against the excessive back-pressure that would result from a high column of absorbent, the upper part of the  $25 \times 330$  mm. tube was expanded into a bulb of approximately 150 ml. capacity. This bulb is not necessary for smaller charges and low percentages of carbonate, but when a charge appreciably beyond a  $\frac{1}{4}$  gram equivalence of calcium carbonate is used, a larger bulb-section should be provided.

The volume of the absorbent in the absorption unit should be commensurate with the expected gas displacement. Usually it is safe to bring the hydroxide to a volume of 200 ml. by the addition of  $\text{CO}_2$ -free water.

### INTERVAL NECESSARY FOR COMPLETE SWEEPING OF LIBERATED CO<sub>2</sub>

Following complete dissolution of the carbonate, it is necessary to propel the liberated gas into the absorption chamber, for although part of the CO<sub>2</sub> reaches the absorption unit during the reflux digestion, the larger part would remain throughout the apparatus. Hence, after water is drawn from the condenser and refluxing ceases, a current of steam is passed through the apparatus to drive the contained air and CO<sub>2</sub> into the ab-

TABLE 1.—*Effect of duration of sweeping of CO<sub>2</sub> recovery from 0.25 gram of calcite<sup>a</sup> by absorption in Ba(OH)<sub>2</sub><sup>b</sup>*

DURATION BEYOND MINIMUM	ANALYTICAL VALUES			
	0.1 N TITRATION		CO <sub>2</sub> EQUIVALENCE	
	ACID	DIFFERENCE	RECOVERY	DEV. FROM THEORETICAL
<i>seconds</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>
0	9.20	50.0	.1100	.0000
0	9.20	50.0	.1100	.0000
30	9.10	50.1	.1102	.0002
30	9.20	50.0	.1100	.0000
60	9.20	50.0	.1100	.0000
60	9.20	50.0	.1100	.0000
90	9.20	50.0	.1100	.0000
90	9.20	50.0	.1100	.0000

<sup>a</sup> Its 0.1100 gram CO<sub>2</sub> equivalence represents 84% of the absorbent capacity of the Ba(OH)<sub>2</sub>.

<sup>b</sup> 60 ml., having a blank titration value of 59.2 ml. of 0.1 normality.

sorption unit. With a steam pressure of 20 mm. of mercury and with passages wide open, the steam reaches that unit in less than one minute. Its arrival is registered by the condensations in the absorption flask and by the heat effect on the side tube of Flask A of Figure 1.

A series of determinations was run to ascertain the duration of the steam flow necessary to assure complete displacement of the CO<sub>2</sub> into the absorbent flask. The minimal duration was the time required for the side-tube to become hot, and this period was prolonged experimentally 30, 60, and 90 seconds. The data given in Table 1 show that complete recovery of the CO<sub>2</sub> was accomplished by the minimal duration of steam sweeping. To make certain that the CO<sub>2</sub> collection is complete in practice, it is only necessary to observe the steam condensation in the upper regions of the absorption flask. The steam flow should not continue unnecessarily, since it will push up the liquid column and cause the escape of some CO<sub>2</sub>.



TABLE 2.—*Effect of carbonate load<sup>a</sup> on CO<sub>2</sub> recoveries by absorption in Ba(OH)<sub>2</sub>*

CARBONATE TAKEN		ABSORBENT TAKEN		CARBONATE LOAD <sup>a</sup>	ANALYTICAL VALUES			
CALCITE	CO <sub>2</sub> VALUE	VOLUME	0.1 N EQUIVA- LENCE		0.1 N TITRATION		CO <sub>2</sub> EQUIVALENCE	
					ACID	DIFFER- ENCE	RECOV- ERY	DEV. FROM THEORETICAL
<i>gram</i>	<i>gram</i>	<i>ml.</i>	<i>ml.</i>	<i>per cent</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>
.0250	.0110	10	12.05	40	7.05	5.00	.0110	.0000
.0250	.0110	10	12.05	40	7.10	4.95	.0109	— .0001
.0500	.0220	10	12.05	80	2.05	10.00	.0220	.0000
.0500	.0220	10	12.05	80	2.15	9.90	.0218	— .0002
.0600	.0264	20	23.70	51	11.70	12.00	.0264	.0000
.0600	.0264	20	23.70	51	11.70	12.00	.0264	.0000
.0800	.0352	20	23.70	68	7.80	15.90	.0350	— .0002
.0800	.0352	20	23.70	68	7.80	15.90	.0350	— .0002
.1000	.0440	20	24.00	83	4.20	19.90	.0438	— .0002
.1000	.0440	20	24.00	83	4.10	20.00	.0440	.0000
.1000	.0440	20	23.70	85	3.70	20.00	.0440	.0000
.1000	.0440	20	23.70	85	3.70	20.00	.0440	.0000
.1100	.0484	20	23.70	93	1.75	21.95	.0483	— .0001
.1100	.0484	20	23.70	93	1.75	21.95	.0483	— .0001
.1150	.0506	20	23.70	97	.80	22.90	.0504	— .0002
.1150	.0506	20	23.70	97	1.00	22.70	.0499	— .0007
.1200	.0528	20	23.70	101	.10	23.60	.0519	— .0009
.1200	.0528	20	23.70	101	.20	23.50	.0517	— .0011
.2400	.1056	40	48.00	100	1.20	46.8	.1030	— .0026
.2400	.1056	40	48.00	100	1.40	46.6	.1025	— .0031

<sup>a</sup> This term is used to connote the percentage of supplied CO<sub>2</sub> in its relation to the normal carbonate capacity, or equivalence, of the absorbent.

### CHOICE OF CO<sub>2</sub> ABSORBENTS

Solutions of barium hydroxide and sodium hydroxide are used in the titrimetric determination of CO<sub>2</sub>. Sodium hydroxide is used alone and with addition of barium chloride. These solutions were considered as to their adaptability for use in the steam-digestion procedures in the following detail:

1. Absorption by a solution of Ba(OH)<sub>2</sub> and its titration to the phenolphthalein end point that corresponds to the pH of the BaCO<sub>3</sub> suspension.
2. Absorption by a solution of NaOH containing BaCl<sub>2</sub> and its titration, as in 1.

3. Absorption by NaOH solution and its titration with phenolphthalein to the bicarbonate end point against a reference solution of pH 8.36.

In the determination of the speed and completeness of CO<sub>2</sub> absorption by the proposed technic, procedures 1 and 3 were used.

#### CO<sub>2</sub> ABSORPTION, AS AFFECTED BY CO<sub>2</sub>-LOAD AND PERIOD OF AGITATION

*With Ba(OH)<sub>2</sub>.*—The charge of calcite was varied from 0.025 to 0.240 gram, with a corresponding range in the volume of barium hydroxide absorbent. In all cases the agitation of the absorbent was exactly 30 seconds,

TABLE 3.—*Effect of period of agitation of the liquid-gaseous phases in the absorption unit on CO<sub>2</sub> recovery, under variant carbonate loads, by absorption in Ba(OH)<sub>2</sub>\**

CARBONATE TAKEN		CARBONATE LOAD	AGITATION PERIOD	ANALYTICAL VALUES			
CALCITE	CO <sub>2</sub> VALUE			0.1 N TITRATION		CO <sub>2</sub> EQUIVALENCE	
				ACID	DIFFERENCE	RECOVERY	DEV. FROM THEORETICAL
<i>gram</i>	<i>gram</i>	<i>per cent</i>	<i>seconds</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>
.1185	—	—	30	0.7	23.0	.0506	— .0015
.1185	—	100	30	0.9	22.8	.0502	— .0019
.1185	.0521	—	60	0.1	23.6	.0519	— .0002
.1185	—	—	60	0.2	23.5	.0517	— .0004
.1185	—	—	120	0.0	23.7	.0521	.0000
.1185	—	—	120	0.0	23.7	.0521	.0000
.1126	—	—	30	1.35	22.35	.0492	— .0003
.1126	.0495	95	30	1.35	22.35	.0492	— .0003
.1126	—	—	60	1.15	22.55	.0496	.0001
.1126	—	—	60	1.20	22.50	.0495	.0000
.1067	—	—	30	2.40	21.30	.0469	.0000
.1067	.0469	90	30	2.40	21.30	.0469	.0000
.1067	—	—	60	2.30	21.40	.0471	.0002
.1067	—	—	60	2.35	21.35	.0470	.0001

\* A constant of 20 ml. of Ba(OH)<sub>2</sub> having a blank titration of 23.7 ml. of 0.1 N acid.

and the titrations were to a uniform pale rose tint. From the data of Table 2 it is evident that complete recoveries were obtained from loads up to 96 per cent.

To obtain more detailed information as to CO<sub>2</sub> recoveries from the maximal load, the volume of the barium hydroxide absorbent was kept constant while the charges of calcite were the respective equivalents of 90, 95, and 100 per cent of the normal carbonate capacity of the hydroxide. Multiple charges were provided to afford duplicate determinations with the several agitation periods of 30, 60, and 120 seconds. The results in

Table 3 show that the full load required more than 60 seconds of agitation to effect complete  $\text{CO}_2$  absorption by the barium hydroxide; that agitation for 60 seconds was sufficient for the 95 per cent load; and that a 30 second period of agitation gave complete recovery of the 90 per cent load.

It is concluded, therefore, that an agitation period of one minute is sufficient for complete absorption of the  $\text{CO}_2$  up to 95 per cent of the barium hydroxide capacity, whereas agitation for 30 seconds is ample for  $\text{CO}_2$  loads of 90 per cent or less.

TABLE 4.—*Effect of carbonate load upon  $\text{CO}_2$  recoveries by absorption in NaOH solution\**

CARBONATE TAKEN		CARBONATE LOAD	ANALYTICAL VALUES			
CALCITE	CO <sub>2</sub> VALUE		0.1 N TITRATION		CO <sub>2</sub> EQUIVALENCE	
			ACID	DIFFERENCE	RECOVERY	DEV. FROM THEORETICAL
<i>gram</i>	<i>gram</i>	<i>per cent</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>
.0500	.0220	97	5.35	4.95	.0218	— .0002
.0500	.0220	—	5.35	4.95	.0218	— .0002
.0600	.0264	116	4.35	5.95	.0262	— .0002
.0600	.0264	—	4.35	5.95	.0262	— .0002
.0700	.0308	136	3.30	7.00	.0308	.0000
.0700	.0308	—	3.40	6.90	.0304	— .0004
.0800	.0352	155	2.35	7.95	.0350	— .0002
.0800	.0352	—	2.40	7.90	.0348	— .0004
.0900	.0396	174	1.35	8.95	.0394	— .0002
.0900	.0396	—	1.40	8.90	.0392	— .0004
.1000	.0440	194	.35	9.95	.0438	— .0002
.1000	.0440	—	.35	9.95	.0438	— .0002

\* A constant of 10 ml. having a blank titration value of 10.30 ml. 0.1 N.

*With NaOH.*—The completeness of absorption of  $\text{CO}_2$  by sodium hydroxide was investigated by use of a constant volume of the alkali and  $\text{CO}_2$  load supplied by charges of calcite between .0500 and .1000 gram. The trapped  $\text{CO}_2$  was made to react with the sodium hydroxide by vigorous agitation of the contents for one minute or longer. The titrations with acid were carried to a rose color to match a like volume of a buffer solution of pH 8.36. The phenolphthalein was a constant. The results of Table 4 demonstrate that complete absorption of  $\text{CO}_2$  occurred for loads as high as 192 per cent of the equivalence of the normal carbonate,  $\text{Na}_2\text{CO}_3$ .

The speed of the  $\text{CO}_2$  absorption was then investigated. As in the previous experiments, a constant of sodium hydroxide was used. Multiple

charges of calcite furnished .022 and .044 gram of  $\text{CO}_2$ , and the agitation periods were 15, 30, and 60 seconds. These two quantities of  $\text{CO}_2$  were 97 per cent and 194 per cent equivalences of the sodium carbonate potential of the sodium hydroxide absorbent and the titrations again were carried to pH 8.36. The results in Table 5 show that with the 97 per cent load the  $\text{CO}_2$  was absorbed almost completely during the 15 second period of agitation, whereas a one-minute period was required for the 194 per cent load.

Since Thomas (6) observed that the inclusion of butyl alcohol imparted a greater effectiveness in  $\text{CO}_2$  absorption, the foregoing series of deter-

TABLE 5.—*Effect of period of agitation of liquid-gaseous phases in absorption unit of  $\text{CO}_2$  recovery under varying carbonate loads in NaOH solution*

CARBONATE TAKEN		CO <sub>2</sub> LOAD*	AGITATION PERIOD	ANALYTICAL VALUES			
CALCITE	CO <sub>2</sub> VALUE			0.1 N TITRATION		CO <sub>2</sub> EQUIVALENCE	
				ACID	DIFFERENCE	RECOVERY	DEV. FROM THEORETICAL
gram	gram	per cent	seconds	ml.	ml.	gram	gram
.0500	.0220	—	15	5.40	4.90	.0216	— .0004
.0500	.0220	—	15	5.35	4.95	.0218	— .0002
.0500	.0220	97	30	5.40	4.90	.0216	— .0004
.0500	.0220	—	30	5.45	4.95	.0218	— .0002
.0500	.0220	—	60	5.35	4.95	.0218	— .0002
.0500	.0220	—	60	5.35	4.95	.0218	— .0002
.1000	.0440	—	15	0.90	9.40	.0414	— .0026
.1000	.0440	—	15	1.05	9.25	.0407	— .0033
.1000	.0440	194	30	0.85	9.45	.0416	— .0024
.1000	.0440	—	30	0.65	9.65	.0425	— .0015
.1000	.0440	—	60	0.40	10.00	.0440	— .0000
.1000	.0440	—	60	0.50	9.90	.0436	— .0004

\* A constant of 10 ml. of NaOH was used; titration of blank was 10.3 ml. of 0.1 N.

minations were duplicated with an introduction of butyl alcohol. The alcohol induced an abundance of foam, but effected no increase in speed of  $\text{CO}_2$  absorption.

### SUMMARY

The apparatus and technic herein described were developed to provide an expeditious and accurate method for the determination of  $\text{CO}_2$  in charges estimated to contain 0.25 gram of calcium carbonate-equivalence, with bulk restricted to 20.0 grams.

A 150 ml. extraction flask was found most suitable as the decomposition vessel. It and its analytical charge were kept one hour in a chamber containing flakes of sodium hydroxide.

The charge is washed down with boiling water, and the flask is connected to a reflux condenser undergoing flushing with a current of steam. Either perchloric or hydrochloric acid of 1+9 dilution is admitted through a separatory funnel, and steam is injected into the suspension from a generator regulated to maintain a pressure of 10 mm. of mercury. Decomposition of mineral carbonates is completed within two minutes of reflux digestion. The water in the condenser jacket is then emptied quickly, and the CO<sub>2</sub> and other gases are swept into the absorption vessel by the steam current. With a pressure of 20 mm. the gases are completely swept from the apparatus in one minute, after which the steam will be seen to condense at the entrance to the absorption flask.

In the absence of hydrogen sulfide, purification of the vapors is accomplished by the interposition of a 50 ml. scrubber bulb containing only water. When the analytical charge contains sulfides, liberated hydrogen sulfide is removed by a series of three bulbs that contain a solution-suspension of silver sulfate maintained at boiling temperature.

The absorption unit comprises a 500 ml. suction flask and a special Pyrex reservoir tube 25×330 mm. This tube is enlarged into a 70 mm. bulb within its upper third. It is fitted into the flask by means of an elastic rubber gasket, which allows the easy sliding of the tube to different elevations within the flask while maintaining a gas-tight joint. A thin film of glycerol serves as a lubricant between the reservoir tube and the rubber gasket. During the decomposition of the charge the lower end of the tube is close to the bottom of the flask and accommodates the absorbent solution displaced by the gases that enter the flask.

After the liberated CO<sub>2</sub> has passed into the absorption flask, the inlet and the Pyrex tube are closed and the tube is raised through the gasket by a twisting motion, so that the lower end of the tube is safely inside the flask. The absorption of the CO<sub>2</sub> within the flask is accomplished by vigorous shaking for one minute. The inside of the tube and the outside of the lower end are rinsed. The tube then is removed and the flask is stoppered. The contained absorbent is titrated by one of the procedures given in the following section of this paper.

The complete operation of decomposition and collection of CO<sub>2</sub> is accomplished within 5 minutes.

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## II. INVESTIGATION OF TITRIMETRIC METHODS

The results presented in the preceding section were obtained by titrations of barium hydroxide-barium carbonate and sodium carbonate systems against phenolphthalein. Certain problems arose in connection with the titration end point. The end points used were relative and did not necessarily represent equivalence values. Since the literature was rather indefinite, and at times even conflicting, it was necessary to study the carbonate titration end point in relation to a number of factors that may affect pH value and the tint imparted by phenolphthalein.

The types of systems considered were: (a) the one resultant from the absorption of  $\text{CO}_2$  in sodium hydroxide solution and the titration of sodium bicarbonate and (b) the one resultant from a like absorption of  $\text{CO}_2$  in sodium hydroxide solution containing barium chloride, and the titration of the excess of hydroxide.

### FACTORS AFFECTING ACCURACY OF DIRECT TITRATION PROCEDURE OF SODIUM CARBONATE TO SODIUM BICARBONATE

A single carbonate titration against phenolphthalein was suggested by Adams (1). This constitutes a special application of the first step of the well-known double titration in alkaline solutions. The chief advantage of the titration to the bicarbonate end point is its simplicity without use of extra reagents and absence of suspensions of basic compounds that are subject to attack by the titrant. This titration is influenced, however, by certain factors that have not been studied in sufficient detail, the chief problem being the probability of escape of  $\text{CO}_2$ . This loss may be appreciable at the equivalence point and is aggravated when the system is shaken or stirred. Furthermore, gaseous  $\text{CO}_2$  may be lost to the space in the absorption unit during agitation of solutions that contain high percentages of bicarbonate. The other problem is that of a true colorimetric end point, and the adoption of a titration technic that will give accurate and reproducible results. First consideration will be given to losses of  $\text{CO}_2$  from variant carbonate-bicarbonate solutions in closed systems.

### COMPUTATION OF ERROR ARISING FROM LOSSES OF $\text{CO}_2$ FROM SODIUM BICARBONATE SOLUTIONS UNDER EXPERIMENTAL CONDITIONS

The relationship between the partial pressure of  $\text{CO}_2$  and the various components of carbonate in solution has been worked out by McCoy (10) and is represented by the equation:  $[2X^2C/kP(1-X)] = K$ , in which the terms have the following significance:

X is the fraction of sodium in the form of bicarbonate;

C is the concentration of sodium in gram-atoms per liter;

k is the molar solubility of  $\text{CO}_2$ , which at  $25^\circ$  and 760 mm. pressure is .0338;

$K$  is the equilibrium constant, found by McCoy to be 5300 for dilute solutions at 25°C.; and

$P$  is the partial pressure of  $\text{CO}_2$ .

The problem was to evaluate the  $\text{CO}_2$  in the vapor phase when carbonate solutions of known initial bicarbonate fractions are shaken in closed containers with definite air space and liquid-volume relationship. Before the foregoing equation can be applied to these data, it is necessary to express the bicarbonate equilibrium value  $X$  in terms of the given initial bicarbonate value  $X_1$ . When a solution of bicarbonate is agitated in a closed container, a certain amount of  $\text{CO}_2$  will be expelled to effect equilibrium with the atmosphere, as a result of the hydrolysis indicated by the equation:  $\text{NaHCO}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NaOH} + \text{H}_2\text{CO}_3$ . According to McCoy, this is followed by the formation of the normal carbonate, as indicated by the equation:  $\text{NaHCO}_3 + \text{NaOH} \rightleftharpoons \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}$ , the net result being  $2\text{NaHCO}_3 \rightleftharpoons \text{Na}_2\text{CO}_3 + \text{H}_2\text{CO}_3$ .

If the fraction of the hydrolyzed sodium bicarbonate be designated as  $A$ , the fraction of  $\text{H}_2\text{CO}_3 = A/2$ . This free  $\text{CO}_2$  in the system is divided between the solution and vapor phases according to Henry's law of solubility of gases:  $P/X = K$ , in which  $P$  is the partial pressure of the gas and  $X$  is its molar solubility. The molar concentration of  $\text{CO}_2$  in solution at 25°C. under partial pressure  $P$ , as given by McCoy, is  $0.0338P$ , measured at 760 mm. Under these conditions the molar concentration of  $\text{CO}_2$  in the vapor phase equals the partial pressure  $P$  divided by the molar volume,  $P/24.45$ , or  $0.0409P$ . To correlate these free  $\text{CO}_2$  fractions with the bicarbonate hydrolysis, the gaseous and the solute  $\text{CO}_2$  have to be combined and expressed in terms of the solution phase. If the volumes of vapor space and solution are equal, a simple addition of the two concentrations given above, i.e.,  $0.0409P + 0.0338P$ , will give the total molar concentration of free  $\text{CO}_2$ , as if it were entirely in the solution phase. When the vapor space and the solution volume are not equal, the vapor phase concentration should be multiplied by the ratio of the vapor volume to the solution volume, and then added to the solution-concentration, as indicated above. In the  $\text{CO}_2$  absorption technic presented here, the writers worked with a solution-volume of 250 ml. and an air space of 250 and 500 ml. Accordingly, the free  $\text{CO}_2$  in these experimental systems will be:  $0.0338P + 0.0409P = 0.0747P$   $M$  for the 250 ml. of air space and  $0.0338P + 0.0818P = 0.1156P$  for the 500 ml. air space. Divided by the sodium concentration of 0.004  $N$  the above values give:

$$\begin{aligned} 0.0747P/0.004 &= 18.7P, \text{ and } A' = 37.4P, \text{ for 250 ml. air space and} \\ 0.1156P/0.004 &= 28.9P, \text{ and } A'' = 57.8P, \text{ for 500 ml. air space.} \end{aligned}$$

The value of  $A$  can be computed similarly for any other concentration of sodium and any ratio of air space to solution-volume. The hydrolyzed

fraction A having been evaluated in terms of P, the equilibrium equation of McCoy may be rearranged and P evaluated in terms of known initial bicarbonate fractions and sodium concentration:

$$P = \frac{2C(X_1 - A)^2}{.0338 \times 5300[1 - (X_1 - A)]}$$

When the above equation was used with insertion of the numerical value of A, the partial pressure of CO<sub>2</sub> and the amount in the vapor phase were computed for various bicarbonate fractions and a sodium concentration of 0.004 N. From the computations given in Table 1, it is apparent

TABLE 1.—*Computed values for partial pressure of CO<sub>2</sub> and CO<sub>2</sub> content of the vapor phase for various values of initial bicarbonate fractions, X<sub>1</sub> of a total sodium concentration, as carbonates, of 0.004 normality*

INITIAL BICARBONATE FRACTION, X <sub>1</sub>	VAPOR SPACE = 250 ML.			VAPOR SPACE = 500 ML.		
	CO <sub>2</sub> PARTIAL PRESSURE	CO <sub>2</sub> VOLUME 25°, 760 MM.	CO <sub>2</sub> CONTENT	CO <sub>2</sub> PARTIAL PRESSURE	CO <sub>2</sub> VOLUME 25°, 760 MM.	CO <sub>2</sub> CONTENT
	atm.	ml.	mg.	atm.	ml.	mg.
1.0	$1.11 \times 10^{-3}$	.28	.49	$8.26 \times 10^{-4}$	.41	.71
.9	$3.15 \times 10^{-4}$	.08	.14	$2.97 \times 10^{-4}$	.15	.28
.8	$1.37 \times 10^{-4}$	.034	.06	$1.35 \times 10^{-4}$	.068	.12
.7	$7.16 \times 10^{-5}$	.018	.03	$7.07 \times 10^{-5}$	.035	.06
.6	$4.00 \times 10^{-5}$	.010	.02	$3.98 \times 10^{-5}$	.020	.03

that the error due to loss of CO<sub>2</sub> in the vapor phase becomes appreciable only after the bicarbonate fraction has become greater than 80 per cent of the sodium content. The theoretical maximal loss of CO<sub>2</sub> from bicarbonate up to 80 per cent of the sodium concentration at 25° C. amounts to only 0.12 mg. of the total .0396 gram content. This loss is 0.3 per cent of the total CO<sub>2</sub> content, and is independent of the total carbonate concentration. Therefore, the maximal error chargeable to this cause would be .0003 gram of CO<sub>2</sub>, when the CO<sub>2</sub> recovered from a .2500-gram charge of limestone is shaken in a 500 ml. air space at 180 per cent carbonate load. A diminution of 10 per cent in this carbonate load brings the computed loss to an insignificant figure.

A decrease in the ratio of air space to solution volume from the value 2 would cause a further decrease in the above-computed loss of CO<sub>2</sub> from the titrated solution.

From these considerations it may be concluded that loss of CO<sub>2</sub> induced by the shaking of bicarbonate solutions to attain equilibrium in closed containers is not enough to preclude the successful utilization of a sodium hydroxide solution as a CO<sub>2</sub> absorbent to the extent of 170 per cent of its sodium carbonate equivalence.



## STANDARDIZATION OF COLORIMETRIC TITRATION END POINT OF SODIUM BICARBONATE

The next step was to implement the direct titration of the absorbed  $\text{CO}_2$  through adoption of a correct and reproducible end point for the bicarbonate titration. In 1881, Warder (18) proposed the use of phenolphthalein in the titration of mixed hydroxide and carbonate solutions. He titrated in the cold to a pale rose tint, and then obtained the residual alkalinity by boiling with an excess of acid. The last-mentioned titration times 2 is equivalent to the original carbonate content of the solution. The more widely known phenolphthalein-methyl orange titration of carbonate, proposed by Brown and Escombe (3), is based on a similar principle. The results by these two procedures are affected by the accuracy of the two end points. The extent of the  $\text{CO}_2$  absorption in standard sodium hydroxide solutions can be determined from the change in titration value with phenolphthalein before and after the  $\text{CO}_2$  absorption, as was pointed out by Adams (1). This technic represents a limited application of the Warder procedure. In his original work Warder (18) showed that correct results are obtained only when the titration is carried to a certain rose tint. The titration procedure thus utilized in the carbonate determination is governed by the accuracy of the indicator for the bicarbonate end point only. Unfortunately, the confusion of ideas regarding the bicarbonate end point and disregard of  $\text{CO}_2$  losses during titration preclude accurate results. After a careful study of the carbonate titration procedures, Kuster (8) concluded that bicarbonate in pure water has a definite rose tint with phenolphthalein. Truog (17) found that variant amounts of indicator are required for variant quantities of carbonate in the titration of sodium carbonate solutions. Schroeder (15) computed the sodium bicarbonate inflection point for various sodium carbonate concentrations upon the basis of the ionization constants of carbonic acid. In discussing the equivalence point of sodium bicarbonate, he observed: "This comes sufficiently close to the colorless point of phenolphthalein ( $\text{pH}$  about 8) so that the end point should be satisfactory." In titrations of 0.01  $N$  and 0.02  $N$  solutions of sodium carbonate by the aid of the hydrogen electrode, Greenfield and Buswell (6) found the inflection point to be close to  $\text{pH}$  8.2. In the titration of carbonate solution in concentrations of .199–1.65 millimole per liter by means of the glass electrode, Cooper (5) found the equivalence point to be at  $\text{pH}$  8.35. In the titration of carbonate in waters by the method of the Association of Official Agricultural Chemists (2) the sodium bicarbonate end point calls for the complete disappearance of the rose color of phenolphthalein. Conversely, Kolthoff (7) gives the  $\text{pH}$  of sodium bicarbonate solution as 8.35, and states that such a solution is not colorless to phenolphthalein until from 3 to 5 per cent excess acid has been added. According to this observation, the titration of carbonate to the colorless end point with phenolphthalein introduces an error of serious

proportions. Furthermore, in the above-reported determinations of sodium bicarbonate equivalence  $pH$  there is scant description of precautionary measures or allowance for escape of  $CO_2$  during the titration of the bicarbonate solution.

#### EXPERIMENTS TO DETERMINE EQUIVALENCE $pH$ OF PURE SODIUM BICARBONATE

Because of the conflict in literature, an attempt was made to determine the  $pH$  for sodium bicarbonate. A series of  $CO_2$ -free aqueous systems was prepared by boiling 325 ml. of distilled water 20 minutes to 250 ml. markings in 500 ml. Pyrex flasks provided with steam-outlet tubes. These were closed immediately before the flasks were removed from the source of heat, and every flask then was connected with a 1×12 inch column of soda-lime and allowed to cool overnight. The tubulated stoppers were replaced with solid stoppers, and the flasks were kept closed except for the brief period necessary for the introduction of the sodium bicarbonate and phenolphthalein. The bicarbonate was added in quantities to make .004, .02, and .10  $M$  concentrations in 250 ml. To minimize the effect of difference in the color of the glass containers, the solutions were made in triplicate. Phenolphthalein was added, and the solutions were mixed by gentle swirling and without shaking. The developed color was compared immediately with borate buffer solutions of the same volume and like indicator concentration. In every instance the initial color was intermediate between that of the buffer solution at  $pH$  between 8.0 and 8.2. The phenolphthalein coloration of the sodium bicarbonate solution deepened upon standing and, after  $\frac{3}{4}$  hour, assumed the tint of buffer  $pH$  8.35. The same tint was also attained upon a 1 minute vigorous shaking of the 250 ml. bicarbonate solution in the 500 ml. closed container. The  $pH$  of the solution was determined also by means of the glass electrode with exclusion of air. At 28° the  $pH$  of a 0.1  $N$  solution of sodium bicarbonate was 8.12; chilled to 18° the  $pH$  was 8.22. Some difficulty was experienced because of instability in readings of the more dilute bicarbonate solutions.

#### CARBONATE TITRATION TECHNIC

It therefore appeared that an accurate end-point tint would be obtained by conducting the titration in a closed vessel with vigorous shaking, and matching against an identical treatment of a pure bicarbonate solution. To facilitate visual comparisons, it was found desirable to have standards of pure sodium bicarbonate solutions with and without 0.5 and 1.0 per cent excesses of sodium hydroxide as guides to the approaching end point. The titration first is matched to "one per cent excess alkali" and then continued cautiously by dropwise addition of acid, with vigorous agitation, until the tint is slightly fainter than that of the "0.5 per cent excess alkali," but not so faint as the one induced by the pure bicarbonate. This

procedure allows for the lag in the reaction of the last portion of the  $\text{CO}_2$  during the titration at the equivalence point. The discrepancy, however, should not be greater than about one-half of the value between the pure bicarbonate and the 0.5 per cent excess of alkali, or about 0.2 per cent of the total determination.

Borate buffers having the proper phenolphthalein tints may be used in lieu of the bicarbonate solutions and would be more permanent.

#### TEST OF THE SODIUM CARBONATE TITRATION PROCEDURE WITH A STANDARD DOLOMITE

A Bureau of Standards dolomite (47.25 per cent  $\text{CO}_2$ ) was used to ascertain the precision and accuracy of the above-described titration procedure. The analytical charges were correlated with volume of the absorbent so that the  $\text{CO}_2$  load was approximately 160 per cent of the sodium hydroxide equivalence. Computations indicated that the 60 per cent excess of  $\text{CO}_2$  above the sodium carbonate equivalence should cause only negligible losses of  $\text{CO}_2$  when the absorption unit is shaken to induce equilibrium. The determinations were carried out in quintets for each series of charges of two magnitudes, one charge being approximately  $2\frac{1}{2}$  times the other.

TABLE 2.— $\text{CO}_2$  recoveries from Bureau of Standards dolomite by absorption in NaOH and titration to  $\text{NaHCO}_3$  end point

DOLOMITE CHARGE	NaOH* TAKEN	0.1 N ACID TITRATION	CO <sub>2</sub> FOUND		DEVIATION FROM AV. PER GRAM	DEVIATION FROM BUREAU OF STANDARDS ANALYSIS
			PER CHARGE	PER GRAM		
<i>gram</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>per cent</i>
.0918	10 ml. solution = 12.17 0.1 N	2.45	.04276	.4658	— .0010	
.0890		2.70	.04167	.4682	.0014	
.0876		2.85	.04101	.4654	— .0014	
.0908		2.52	.04246	.4676	.0008	
.0906		2.53	.04242	.4669	.0001	
Average				.4668	.00094	— .52
.2176	25 ml. solution = 30.55 0.1 N	7.37	.10182	.4671	— .0005	
.2206		6.92	.10397	.4668	— .0008	
.2230		6.80	.10450	.4686	.0010	
.2257		6.50	.10582	.4689	.0013	
.2256		6.62	.10529	.4667	— .0009	
Average				.4676	.00090	— .44
Weight av. for 10 determinations				.4673	.00091	— .47
Deviation in percentage of total $\text{CO}_2$						— 1.0

\* The  $\text{CO}_2$  load in all experiments of this table was approximately 160% of the NaOH equivalence.

The values in the third column in Table 2 are those from which the  $\text{CO}_2$  recoveries are calculated. These values were subtracted from the initial titration values of the absorbent, as given in the second column, and multiplied by 4.4 to derive the results for  $\text{CO}_2$  per charge in the fourth column. The values in the fifth column were computed to facilitate comparisons of results from the variant quantities of carbonate used in the determinations. The mean of values for  $\text{CO}_2$  per gram from the smaller charges differs from the mean of the values for larger charges by .0008 gram of  $\text{CO}_2$ . Translated in terms of determined  $\text{CO}_2$ , this difference amounts to less than .0001 gram of  $\text{CO}_2$  for the smaller charge and about .0002 gram on the large charge. The average deviations are practically identical for the two series of results and amount to .0009 gram of  $\text{CO}_2$  per gram of dolomite. When a mean of ten determinations is used, the precision is given by the mean deviation of .00091 gram in 0.4679 gram of  $\text{CO}_2$ . Hence, the mean error in a single determination is  $\pm 2$  in 1000. The accuracy of the analysis in relation to the Bureau of Standards value is given in the last column of the table. There appears to be a mean deficiency of 0.47 per cent of  $\text{CO}_2$ , or 1 per cent of the total  $\text{CO}_2$  content, after correction of tabulated results for 0.1 per cent moisture in the analytical charge. This deviation constitutes a systematic error of minus 1 per cent and might not be considered a serious error in an ordinary analysis. In view of the high precision of the results, however, this deviation merits further study. Of the two possible sources of error, one may reside in the titration and the other in the incompleteness of the  $\text{CO}_2$  absorption within the prescribed period of shaking.

#### TEST OF SODIUM BICARBONATE TITRATION END POINT BY DIRECT TITRATION OF PURE SODIUM CARBONATE

To make a clear-cut test of the accuracy of the titration of sodium carbonate as outlined above, titrations were performed on pure sodium carbonate freshly prepared from high-grade sodium bicarbonate by heating 3 hours at  $270^\circ$ . The acid was first standardized by titration against 5 weighings of approximately 0.250 gram of sodium carbonate to pH of 3.80 in a volume of about 50 ml. by the aid of the glass electrode. The results of this titration are given in Table 3. Five weighings of approximately .2650 gram of sodium carbonate then were dissolved in 225 ml. of  $\text{CO}_2$ -free water, prepared, and titrated in manner described, and with the same acid in the absence of  $\text{CO}_2$ . The values given in Table 4 indicate that the sodium carbonate titration *per se* may be accomplished with a high degree of precision by the use of the adopted titration technic against phenolphthalein. The mean deviation for 5 determinations is 2 in 1000. The mean error of this titration in relation to the half-value of the potentiometric determination of the same sodium carbonate (Table 3) is  $-2.4$  per 1000. The minus error would cause a 0.2 per cent positive error in the

TABLE 3.—*Titration of  $\text{Na}_2\text{CO}_3$  to  $\text{H}_2\text{CO}_3$  potentiometrically with glass electrode to pH 3.8*

$\text{Na}_2\text{CO}_3$ CHARGE	ACID* USED PER CHARGE	ACID USED PER GRAM	DEVIATION FROM AVERAGE PER GRAM
<i>gram</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
.2533	47.66	188.2	.1
.2380	44.78	188.2	.1
.2304	43.32	188.2	.1
.2477	45.41	187.9	-.2
.2511	47.20	188.0	-.1
Average	—	188.1	.12

Titration error &lt;1 in 1000

\* Computed concentration:  $188.7/188.1 = 0.10032\text{ N}$ .TABLE 4.—*Titration of  $\text{Na}_2\text{CO}_3$  to  $\text{NaHCO}_3$  by proposed technic with phenolphthalein as indicator*

CHARGE	ACID USED	ACID PER GRAM	DEVIATION PER GRAM	DEVIATION FROM POTENTIOMETRIC VALUE PER GRAM
<i>gram</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
.2671	25.05	93.6	-.24	-.45
.2775	25.62	94.0	.16	-.05
.2887	27.02	93.6	-.24	-.45
.2487	23.37	94.1	.26	.05
.2603	24.45	93.9	.06	-.15
Average		93.84	.19	-.23
Titration error			2 per 1000	-2.4 in 1000

carbonate determination by this titration. This slight deviation represents a much higher degree of precision and accuracy for this type of titration than that hitherto reported.

#### SUMMARY OF FINDINGS ON THE DIRECT SODIUM CARBONATE TITRATION PROCEDURE

The titration of sodium carbonate to the colorless "phenolphthalein end point" may produce errors as high as 5 per cent, as governed by the conditions of the titration. The transition point for sodium bicarbonate as reported in the literature is from 8.35 to 8.40 pH. The findings of the writers, have shown, however, that such values could be obtained only after the sodium bicarbonate has been shaken or allowed to stand for some

time in a closed container with considerable air space. The  $pH$  value of a solution of pure sodium bicarbonate immediately upon dissolution in  $CO_2$ -free water is approximately 8.15, and rises quickly upon standing, even in closed containers incompletely filled.

Exposure to atmosphere and variation in phenolphthalein concentration are the chief sources of error in this type of titration. When the bicarbonate fraction in sodium carbonate-sodium bicarbonate solution represents between 60 and 70 per cent of the total sodium content, the loss of  $CO_2$  to the air in the container with limited air space is negligible.

Titration of sodium carbonate solutions within  $\pm .2$  per cent accuracy can be made in a closed container when the phenolphthalein end point is matched to the rose tint obtained from an 8.40  $pH$  buffer control solution of identical volume and indicator concentration and in same type of flask.

#### TITRATION OF HYDROXIDE IN PRESENCE OF BARIUM CARBONATE SUSPENSIONS

When  $CO_2$  is absorbed by a solution of either barium hydroxide, or sodium hydroxide with subsequent addition of barium chloride, the  $CO_2$  is precipitated as barium carbonate. The titration of the excess of the hydroxide against phenolphthalein may be performed either directly, in the presence of the suspension of barium carbonate, or after clarification by either settling or filtration. Partridge and Schroeder (11) concluded that greater accuracy is attained when the barium carbonate is left in the system to be titrated. In nearly all the investigations the "phenolphthalein end point" is identified with complete decolorization of the indicator. This titration end point was adopted by Winkler (8), who originated the procedure of carbonate precipitation from hydroxides by means of barium chloride, and was followed by other investigators (8, 10, 17).

A critical examination of the literature as to titration of sodium hydroxide in the presence of a precipitate of barium carbonate reveals that such end point cannot be relied upon to give accurate evaluation of the hydroxide-carbonate relationship. Cain (4) appears to be the first to call attention to the error resultant from the hydrolysis of the barium carbonate. In an attempt to correct the error introduced by the solubility of barium carbonate at the phenolphthalein titration end point, Schollenberger (13) suggested a titration to a  $pH$  of approximately 10 against thymolphthalein. Apparently, he concluded later (14) that neither the phenolphthalein end point nor the titration with thymolphthalein can be expected to give stoichiometric titration values by his carbonate procedure since he wrote as follows: "For the highest accuracy it is best to standardize the acid against a sample of pure  $CaCO_3$  carried through the procedure" (14).

Because of the early adoption of the Winkler procedure in testing for incidence of sodium oxide in sodium carbonate utilized as a primary standard, the sodium carbonate-barium chloride system has been subjected to more thorough investigation than has the alternative  $\text{CO}_2$  absorption system of barium hydroxide-barium carbonate. Sorensen and Anderson (16) arrived at the following conclusions regarding the Winkler procedure: (a) Precipitation of barium carbonate by the addition of barium chloride in the cold results in an alkaline solution, the alkalinity of which is attributed to the occlusion of bicarbonate in the barium carbonate precipitate; (b) most accurate results are obtained when the precipitation is from a warmed solution; (c) in the presence of an excess of hydroxide the barium carbonate carried hydroxy-carbonates in proportion to both bulk of precipitate and concentration of the hydroxide. The conclusions of Le Blanc (9), however, are in direct conflict with those of Sorensen and Anderson. Upon precipitation of normal carbonate by addition of barium chloride, he found the reactions to be acidic to phenolphthalein in both hot and cold solutions, and attributed this to the dragging down of hydroxy-carbonates during the barium carbonate precipitation. Schmitt (12) studied the methods for testing the purity of sodium carbonate preparations, the procedure of Sorensen and Anderson being included. His findings accord with those of Le Blanc and are in conflict with those of Sorensen and Anderson. Using the solubility constant of barium carbonate and dissociation constants of carbonic acid, Schmitt made theoretical computations of the  $\text{OH}^-$ -ion concentration of barium carbonate suspension in pure water and with an excess of barium chloride. He concluded that excess of barium chloride of at least 0.03  $M$  is required to repress the hydrolysis of barium carbonate to the colorless point of phenolphthalein. It is not apparent how Schmitt harmonized his experimental findings with his own computations. His figures for  $\text{OH}^-$ -ion concentration of pure barium carbonate solution are  $2.5 \times 10^{-4}$  and for  $\text{OH}^-$ -ion concentration in the presence of 0.033  $M$  barium chloride— $1.3 \times 10^{-5}$ . In terms of  $\text{H}^+$ -ion, these values are equivalent to  $pH$  of 10.40 and 9.11 for the respective conditions. Even with the allowance for the low phenolphthalein concentration used by Schmitt, it is not apparent how  $pH$  of 9.11 could be identified with the colorless point of this indicator.

The conflicting viewpoints presented by the citations as to the nature of the precipitation of pure carbonate by additive barium chloride may be accounted for by a lack of uniformity in experimentation, with particular regard to indicator concentration and  $\text{CO}_2$  contamination. There is also a lack of reliable information as to the  $pH$  of systems containing suspensions of barium carbonate in the presence of variant excesses of barium chloride for guidance for the titration of the end point of sodium hydroxide in such systems.

## EXPERIMENTS WITH PURE SODIUM CARBONATE

To obtain information on the  $pH$  value of the barium chloride-sodium chloride system and to provide specific suspensions as standards of comparison for titrations of unknowns, a series of sodium carbonate solutions was prepared as follows: Approximately 325 ml. of ordinary distilled water was introduced into 500 ml. Pyrex suction flasks, calibrated also for 250 ml. The side tubes were closed, and into the mouths of the flasks were fitted rubber stoppers that carried Pyrex glass goosenecks to serve as steam outlets. The water was boiled vigorously over Bunsen burners about 20 minutes to a volume of 250 ml. Then, and while the water was boiling vigorously, each stoppered flask was held together in one hand by means of a towel and the steam-outlet tube was closed with the other by means of a pinch clamp. The flasks were then removed from the flame and connected quickly through outlet tubes to large soda-lime tubes. The clamp was removed, and the flasks were placed near an open window. After cooling, preferably overnight, variant quantities of sodium carbonate in the range of 2.5–250 mg. were introduced quickly. The sodium carbonate was dissolved with slight swirling of contents, and 12.5 ml. of a neutral 2  $N$  solution of barium chloride was introduced. To the solution then was added 0.25 ml. of 1 per cent solution of phenolphthalein, and the tubulated stoppers were replaced with solid ones. After the flasks had been shaken vigorously a few seconds, they were placed over a white surface for observation as to indicator tint and precipitation characteristics.

PHENOLPHTHALEIN TINT AND  $pH$  OF BARIUM CHLORIDE-SODIUM CARBONATE REACTION SYSTEM

Observations of the indicator tints of the various sodium carbonate precipitations were made after 10 minutes and 1, 2, and 24 hours. Whenever possible, the tints were correlated with  $pH$  standards by comparison with a series of buffer solutions of like volume and indicator concentration contained in similar flasks. The normality of the sodium carbonate solutions ranged from .0001 to .01 before precipitation, whereas the barium chloride concentration was a constant of 0.1  $N$ . Since the sodium carbonate was of high purity, the  $pH$  of the several suspensions should have  $pH$  corresponding to that of a saturated solution of barium carbonate, altered only by the Ba-ion concentration of the barium chloride and influenced to some extent by the Na-ions of the generated sodium chloride. During the first 10 minutes all the suspensions had a  $pH$  close to 9. The indicator color faded considerably during the first hour and after 2 hours the  $pH$  of the 2.5, 5.0, and 10 mg. sodium carbonate systems was about 8.60. The  $pH$  of the higher sodium carbonate concentrations could not be determined, since the barium carbonate precipitate formed instantly and remained in partly colloidal suspension. The precipitates in



the lower range of sodium carbonate concentrations were formed gradually in a fine suspension which settled out completely within 2 hours. Only in this lower range of sodium carbonate concentration could the *pH* of the precipitation system be determined by colorimetric comparison with the buffer solutions. After 24 hours the 2.5 and 5.0 mg. systems showed *pH* of 8.50, whereas the 10 and 50 mg. systems appeared to have *pH* of 8.45. The systems that contained 100 mg. and 250 mg. of sodium carbonate did not settle to clarity and were estimated to be at *pH* of 8.3–8.4. The foregoing observations lead to the conclusion that in the heavier barium carbonate precipitations there is a slight hydroxyl-ion precipitation with resultant diminution in *pH* of the solution phase. It should be recognized, however, that the actual differences in OH-ion concentration, as affected by the above-cited differences in *pH*, are insignificant. This was established by the fact that the addition of a single drop of 0.1 *N* acid wiped out the extreme *pH* differences in the above series.

#### TEST OF TITRATION PROCEDURE IN PRESENCE OF BARIUM CARBONATE BY USE OF STANDARD DOLOMITE

To establish the accuracy and the precision of the titration in the presence of barium carbonate as outlined above, charges of Bureau of Standards dolomite were weighed to yield 90 per cent of the CO<sub>2</sub> equivalence of the 50 ml. and 25 ml. aliquots of sodium hydroxide absorbent. The determinations were carried out as prescribed under the proposed procedure, and titrations were made by matching the phenolphthalein tint of the barium carbonate suspensions derived from pure sodium carbonate + barium chloride. The results of 15 such determinations are given in Table 5. The high degree of precision and the attained agreement with the Bureau of Standards analysis leave little to be desired. Further verification of the precision of the procedure was afforded by the close concordance attained in tests as to reproducibility of results over extended periods.

#### SUMMARY—TITRATION OF HYDROXIDE IN PRESENCE OF BARIUM CARBONATE

It is concluded that the proper titration end point in an aqueous system containing barium carbonate and barium chloride of 0.1 normality should have a *pH* between 8.50 and 8.60. Beyond the statement that the titration equivalence of the hydroxide in presence of barium carbonate is distinctly alkaline to phenolphthalein, this conclusion is not of particular help in the analytical titration in the presence of barium carbonate precipitate, since the corresponding tint of phenolphthalein at the stipulated *pH* will vary with the bulk of the barium carbonate. The greater the quantity of barium carbonate suspension, the paler will be the tint of the system at *pH* of 8.6. This effect on phenolphthalein tint at any given *pH*

should not be confused with the influence that the bulk of precipitate exerts upon the actual pH of the system, because of slight hydroxide absorption. It follows that to obtain an accurate titration end point of hydroxide in the presence of barium carbonate suspensions, it is essential to have a series of varying concentrations of pure sodium carbonate precipi-

TABLE 5.—*Recovery of CO<sub>2</sub> from Bureau of Standards dolomite by absorption in NaOH and precipitation with BaCl<sub>2</sub>*

DOLOMITE CHARGE	.1003 N ACID TITRATION			CO <sub>2</sub> EQUIVALENCE OF NaOH USED		DEVIATION FROM AVERAGE PER GRAM	DEVIATION FROM BUREAU OF STANDARDS ANALYSIS
	NaOH TAKEN	BACK TITRATION	NaOH USED	PER CHARGE	PER GRAM		
<i>gram</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>per cent</i>
.2538		6.55	54.50	.1202	.4736	.0007	
.2572	50 ml. sol.	5.95	55.10	.1215	.4724	— .0005	
.2558	≈ 61.05 ml.	6.00	55.05	.1214	.4746	.0017	
.2727	acid	2.70	58.35	.1288	.4723	— .0006	
.2725		2.75	58.30	.1286	.4716	— .0013	
Average of run 7/16				.4729		± .0010	+ .04
.1096		7.02	23.43	.0517	.4717	— .0008	
.1218	25 ml. sol.	4.36	26.09	.0576	.4729	.0004	
.1150	≈ 30.45 ml.	5.80	24.65	.0544	.4722	— .0003	
.1233	acid	4.04	26.41	.0583	.4728	.0003	
.1210		4.50	25.95	.0572	.4727	.0002	
Average of run 7/17				.4725		± .0004	± .00
.2614		5.10	55.90	.1234	.4719	— .0005	
.2530	50 ml. sol.	6.75	54.25	.1197	.4732	.0008	
.2533	≈ 61.05 ml.	6.80	54.20	.1196	.4722	— .0002	
.2626	acid	4.95	56.05	.1237	.4710	— .0014	
.2557		6.10	54.90	.1211	.4738	.0014	
Average of run 8/7				.4724		± .0009	— .01
Average of 15 determinations				.4726		± .0008	+ .01

tated with barium chloride as standards for variant unknown carbonate concentrations. In practice, however, two such standards probably would suffice. These would be obtained by precipitations of 10 mg. and 100 mg. of sodium carbonate in 250 ml. of water by the same quantity of barium chloride and with the indicator concentration identical with that of the unknowns. Selection of reference standard should be governed by comparison of the translucency of the unknown with that of the several reference suspensions.

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## III. PROCEDURE FOR INORGANIC MATERIALS

Based on work described in Parts I and II, the proposed procedure was adapted to the determination of the  $\text{CO}_2$  content of mineral carbonates, baking powders, and miscellaneous inorganics.\*

## REAGENTS

*Acid solution.*—Dilute solution (1+9) of either  $\text{HCl}$  or  $\text{HClO}_4$ .

*Absorbent solution.*—Prepare ca. 0.12 *N*  $\text{NaOH}$  by taking 7 ml. of concentrated  $\text{NaOH}$  (1+1) per liter. Preserve in a Pyrex bottle provided with automatic 10 and 25 ml. Pyrex pipets and with protective soda-lime tubes.

*Barium chloride solution.*—Dissolve 306 grams of  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  per liter. Connect the container with a 10 ml. delivery pipet and protect from  $\text{CO}_2$ .

*Standard acid.*—Prepare exactly 0.1 *N*  $\text{HCl}$ .

*Phenolphthalein indicator.*—1% solution in ethyl alcohol.

## APPARATUS

*Apparatus and tubing.*—Use Pyrex glass. The amber type tubing, smooth-finished,  $\frac{1}{8}$  inch with 1/16 inch wall thickness, was found most suitable for steam flow connections. The four functional units of the apparatus are illustrated in Figure 1.

*Power unit.*—Consists of a 3 liter flask, SG, containing slightly acidified distilled water; a mercury manometer, M; an outlet tube provided with a pressure release valve, P. Heat is supplied by a Tirrill gas burner.

*Decomposition unit.*—Consists of a 150 ml. extraction flask, D; an 8 inch condenser, C; a 30 ml. separatory funnel, F, and steam inlets provided with clamps  $P_1$  and  $P_2$ . The stopper CS is held firmly on the tip of C to support funnel F and inlet tube  $P_1$ . The supporting clamps  $S_1$ ,  $S_2$ , and  $S_3$  are fastened to the same support-rod so that the several parts are held as a unit.  $C_1$  and  $C_2$  are day clamps for quick discharge of water from the condenser jacket.

\* The procedure proposed for soils is given in Part IV.

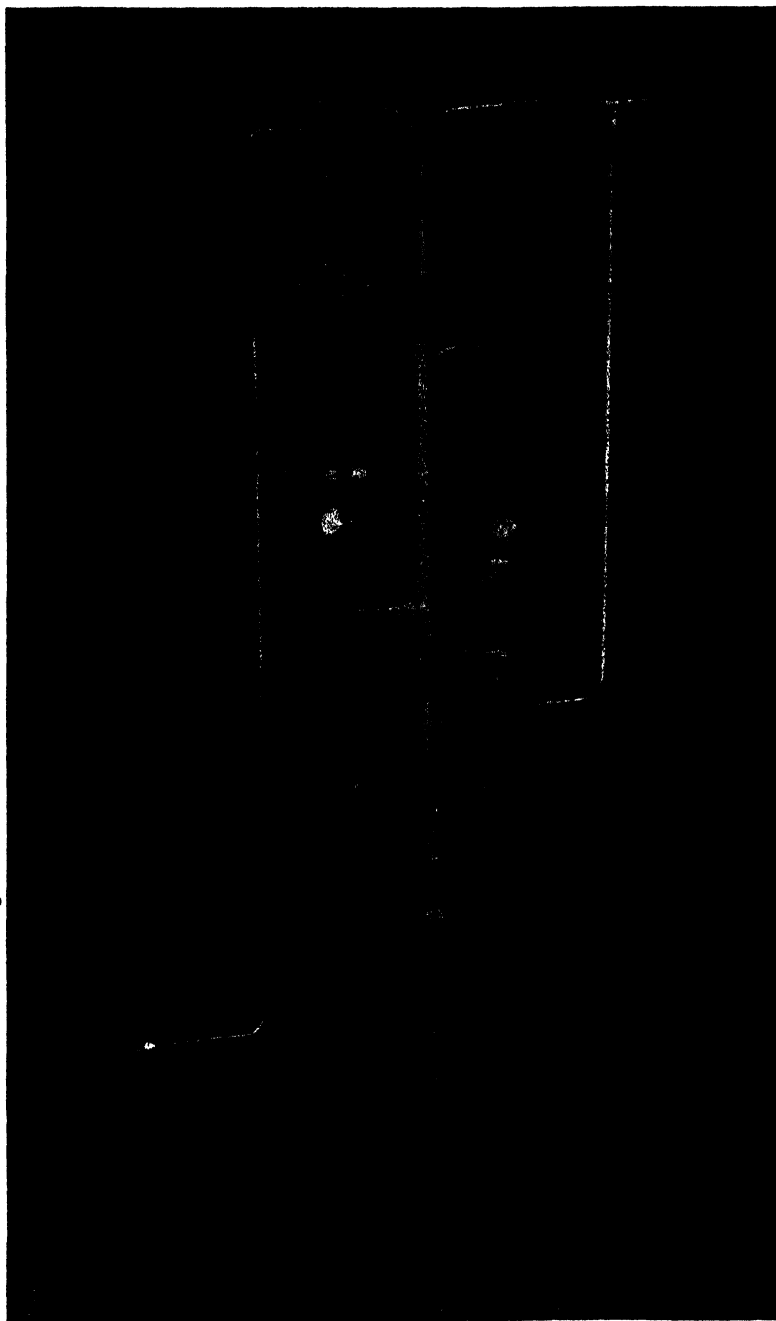


FIG. 2.—DECOMPOSITION UNIT WITH SIMPLIFIED PURIFICATION TRAIN FOR CARBONATE DETERMINATION IN ABSENCE OF  $H_2S$

**Purification unit.**—Consists of three 50 ml. extraction flasks,  $E_1$ ,  $E_2$ , and  $E_3$ . The flask  $E_2$  contains 20 ml. of a suspension of  $AgSO_4$  in dilute  $H_2SO_4$  (1+19).  $E_2$  serves to trap any splash of acid from  $E_3$ , and  $E_1$  serves to catch the liquid that backs up from  $E_2$ . The flasks are held immersed in a solution of  $CaCl_2$  (550 grams in 1000 ml. of water) by means of a wooden yoke-type clamp that rests on the edges of the 170 mm. crystallizing dish, B. A small burner serves to maintain the bath at a temperature of ca. 110°C.

**Scrubber bulb.**—An alternative to the purification unit in the absence of  $H_2S$ . Consists of a single flask, E, which contains initially ca. 10 ml. of  $H_2O$ . It has a syphon tube for withdrawal or recharging with hot water.

**Absorption unit.**—Consists of a 500 ml. filtering flask A and the specially designed tube R. An airtight joint between A and R is attained by means of a rubber gasket, RG, made from No. 7 amber rubber stopper having a 25 mm. bore and lubricated with a thin film of glycerol. The 2-holed stopper over R is provided with a soda-lime tube, SL, and an inlet tube with screw clamp, I. The glass tubing, CT, which is attached to the side tube on flask A is of sufficient taper and length to make a broad tight joint with the rubber tubing on the terminal of the steam line. The screw clamps  $P_1$  and  $P_2$  serve to close the absorption apparatus and steam line, respectively.

**Titration cap.**—Consists of a 10 mm. section of a 2-holed No. 8 stopper provided with a skirt of 2 inch Gooch tubing, G, to slip over the neck of flask A. The tubing is held by a wire fitted into a groove around the rubber disk. One hole serves as the entrance for the buret tip; the other holds a clamp for the instantaneous release of pressure following the addition of acid.

#### DETERMINATION

**Preparation of charge.**—Grind the material to fineness necessary for uniformity in sampling. Weigh charge to contain ca. 0.25 gram of  $CaCO_3$ , the total charge, however, not to exceed 20 grams. Transfer charge into the decomposition flask and keep in a  $CO_2$ -free atmosphere 1 hour before analysis.

**Purification of apparatus.**—Prepare a supply of boiling distilled water and apply heat to the steam generator and the purifying train. Maintain the bath of the purifying unit at ca. 110°C. Connect empty flask D at CS; see that condenser jacket is empty; close  $P_1$ ,  $P_2$ ; open  $P_3$ , and allow steam to flow through  $P_4$ . Adjust flame under SG to a manometer indication of 10 mm. and continue steam flow 15 minutes; close  $P_4$ , open  $P_3$ , and remove flask D. Close  $P_2$  and let steam pass from  $P_3$  through C until the apparatus is ready to be used.

Purge the  $CO_2$  in the absorption unit by evacuating at I and replacement with  $CO_2$ -free air through  $P_1$ . Introduce through I either 10, 25 or 50 ml. of the absorbent according to expectancy of  $CO_2$ . Wash down tubing I and introduce enough  $CO_2$ -free  $H_2O$  to reach about two-thirds up the bulge of the tube and close. To assure airtight joint, note height of column of liquid for a brief period and see that the liquid does not recede. Make a temporary connection between SL and CT with tubing; open  $P_1$  to allow liquid in R to descend into A, and close.

**Decomposition of sample and collection of  $CO_2$ .**—Connect the absorption unit by slipping rubber tubing  $P_4$  over the connector CT. Introduce the acid (1+9) into F. Fill the steam inlet tube with water by dipping it into a beaker containing  $CO_2$ -free water while quickly opening and closing  $P_2$ . Remove flask from the  $CO_2$ -free chamber, rinse with 40 ml. of boiling water,\* and connect tightly with CS. Close  $P_2$  and admit acid slowly into D, leaving the stem filled above stopcock. Open  $P_1$  and start circulation through the condenser. When the manometer registers equalization in pressure, open  $P_4$  and  $P_3$ . Continue the reflex digestion for 2 minutes, during which

\* This step is omitted with samples, like baking powder, which are decomposed on wetting.

the steam pressure is regulated by the manipulation of  $P_1$  so that the condensate surge is held within the lower half of the condenser. Give D an occasional vigorous swirling motion to wash down any particles thrown up above the liquid zone during the digestion. This also serves to diminish foaming encountered with some materials at the early stage of the digestion. After two minutes of digestion close  $P_1$ ; cut off water supply and discharge water from the jacket of the condenser by pinching  $C_1$  and  $C_2$  simultaneously. Continue steam passage until the juncture of flask A and side arm becomes too hot to hold. Open  $P_3$  and continue steam passage only 30 seconds. Close  $P_3$  and  $P_4$ ; remove D from apparatus and rinse stopper CS. Close  $P_2$  and disconnect absorption unit. This operation is facilitated by placing a drop or two of water on the  $P_4$ -CT junction while pinching the rubber tubing  $P_4$ .

#### CO<sub>2</sub> ABSORPTION AND TITRATION

(a) *Titration to NaHCO<sub>3</sub>*.—Allow the CO<sub>2</sub> absorption unit to come to room temperature. Remove cap RC; introduce 0.25 ml. of the phenolphthalein solution, and then stopper. While pressing firmly against RG with one hand, raise tube R with a twisting motion so that its lower end is just above the side tube in flask A. Hold the parts together tightly with both hands and shake vigorously 30 seconds to assure that the gaseous CO<sub>2</sub> comes into intimate contact with the absorbent. Remove stopper and rinse it and the inside of the tube with CO<sub>2</sub>-free water. Then remove tube and gasket; wash the outside of the lower end of the tube, limiting volume to 250 ml.; and either titrate immediately or stopper.

Slip the titration cap over the opening of flask A and introduce the tip of the buret that contains the standard acid so that the acid discharge can be seen below the rubber tubing. With constant swirling of contents, titrate *slowly* to a pale rose tint corresponding to that of a buffer solution of like volume and identical indicator concentration in a similar flask at pH of 8.4. Subtract this titration from the "blank" value obtained in the same manner on the unknown. The difference in ml. of 0.1 N acid, multiplied by 0.0044, gives grams of CO<sub>2</sub>.

(b) *Titration of hydroxide in presence of BaCO<sub>3</sub>*.—Introduce 10 ml. of the BaCl<sub>2</sub> solution through I. Remove cap RC and introduce indicator; stopper, and proceed with shaking and removal of tube R and then titrate as directed under (a), except to use BaCO<sub>3</sub> suspensions as titration references. These suspensions are prepared by dissolving 0.010 and 0.100 gram charges of pure Na<sub>2</sub>CO<sub>3</sub> in identical volume of CO<sub>2</sub> free water introduced into the CO<sub>2</sub> free absorption flask. The suspensions should be allowed to stand 2 hours before being used as standards. They are good for 3 days. The quantity of indicator and of BaCl<sub>2</sub> should be the same as that used in the analytical determination.

Match the titration against that of the reference suspension whose bulk conforms most nearly to that of the unknown. Subtract this titration value from the blank titration, obtained in the same manner as for the unknown. The difference in ml. of 0.1 N acid, multiplied by .0022, gives grams of CO<sub>2</sub>.

#### IV. ADAPTATION FOR SOIL CARBONATES

The accurate determination of the carbonate content of soils is essential to various problems in soil research, notably studies as to the progression in the decomposition of limestone incorporations and in the carbonatation of the hydroxides and silicates of calcium and magnesium. The chief requisites for that determination are the complete decomposition of component carbonates and a minimum of CO<sub>2</sub> generated through

disintegration of organic matter content. Those two considerations have been stressed in methods developed during the past two decades.

MacIntire and Willis (4) demonstrated that  $\text{CO}_2$  is evolved when carbonate-free soils are digested with boiling dilute hydrochloric acid. To minimize this effect, they proposed a method (5) that stipulated aspiration under continuous agitation with dilute hydrochloric acid at room temperature. Prolonged digestion was required to assure the analytical decomposition of certain additive dolomites by this procedure, unless the dolomited soil was pulverized. Schollenberger (6) added ferrous chloride to dilute hydrochloric acid as an antioxidant and expedited the carbonate decomposition by boiling in vacuo at  $27^\circ\text{--}30^\circ\text{C}$ . His procedure stipulates that samples be ground to minus 100-mesh and that the digestion period be 45 minutes to assure complete dissolution of dolomitic limestones, with an 8-minute sweeping period.

With the dual objective of checking the oxidative activities and expediting the dissolution of additive dolomitic limestone, one of the writers (7) proposed that the MacIntire-Willis procedure be modified by (a) the inclusion of stannous chloride, (b) an abbreviated boiling period, and (c) absorption of  $\text{CO}_2$  in ascarite bulbs. In the analysis of non-calcareous soils and those of nugatory carbonate content, however, satisfactory reproducibility of results was not attained. Alexander and Byers (1) conducted a critical study of soil carbonate determinations. They concluded that a 15 minute boiling digestion in 12 per cent hydrochloric acid (1+2) is satisfactory, although their results showed an average  $\text{CO}_2$  evolution of .082 per cent from six acidic soils. When aspirated one hour with 5 per cent hydrochloric acid, the same soils yielded a mean  $\text{CO}_2$  evolution of .028 per cent, which was attributed to meager incidences of carbonates.

The present study demonstrated that, with steam, 0.5 *N* hydrochloric acid effects complete decomposition of analytical charges of even the most resistant dolomites in less than 2 minutes, and that complete removal of the liberated  $\text{CO}_2$  is accomplished by a steam sweep of  $1\frac{1}{2}$  minutes. Additional points considered were evolution of  $\text{CO}_2$  induced by action of dilute hydrochloric acid upon the soil organic matter during the brief digestion, the retardation of that action by means of antioxidants, and the utility of the proposed technic in its relation to other  $\text{CO}_2$  procedures.

#### $\text{CO}_2$ EVOLUTION FROM ORGANIC MATTER AND THE INFLUENCE OF ANTIOXIDANTS

The extent of the decomposition of organic matter by the liberative acid was determined by refluxed hydrochloric acid digestions of a number of acidic soils. Since the soils selected were highly acidic, they were presumed to be devoid of mineral carbonates. The Bolton and the Tellico silt loams possessed marked inherent oxidative capacity. Their content of iron and manganese was high, whereas that of the gray Hartsells sandy

loam and of the Clarksville silt loam was low. Organic matter content was fairly uniform at about 2.5 per cent. A strongly acidic peat from Minnesota also was included. The  $\text{CO}_2$  evolution was determined after 2 and 5 minute reflux digestions with dilute hydrochloric acid, alone and with inclusions of stannous chloride and also with ferrous sulfate. These two periods can be considered as representing extremes in steam passage.

Results are indicated by the data in Table 1. Those in the first column show that the evolutions of  $\text{CO}_2$  from *acid alone* were influenced by the sesquioxide contents of the soils. The maximal  $\text{CO}_2$  evolution of .08 per cent came from the 2-minute digestion of the reddish-brown Tellico and Bolton silt loams, whereas the evolutions from the light colored Hartsells and Clarksville soils were only 0.013 and 0.033 per cent, respectively. But, with an 80 per cent content of organic matter, the peat yielded a  $\text{CO}_2$  evolution of only 0.055 per cent, whereas the clay subsoil of meager organic matter content gave a  $\text{CO}_2$  evolution of 0.005 per cent. When the refluxed digestions of the soils with hydrochloric acid alone were extended to 5 minutes the 3-minute extension caused a mean increase of 40 per cent in  $\text{CO}_2$  evolutions. In contrast, this additional digestion caused no increase in the  $\text{CO}_2$  from the subsoil. It is evident that a small percentage of  $\text{CO}_2$  may be evolved from acidic soils, even when the factor of the decomposition of organic matter is virtually eliminated. Since the soil-waters of acidic soils invariably contain some bicarbonate of calcium, the  $\text{CO}_2$  liberated from the subsoil during the straight hydrochloric acid digestion may be attributed to minute quantities of calcium carbonate thrown down during the drying of the soil.

The data in Table 1 show that the most noticeable antioxidant effect upon  $\text{CO}_2$  evolution occurred in the brownish soils, such as the Bolton and Tellico silt loams, which are characterized by high content of oxides of iron and manganese and low content of organic matter. These data also show that the antioxidants did not repress the evolution of  $\text{CO}_2$  from either the peat or the subsoil. This lack of repressive effect on  $\text{CO}_2$  evolution from the clay is explained readily as being due to a virtual absence of organic matter, whereas the lack of a repressive effect upon the decomposition of the peat organics may be ascribed to the absence of those mineral compounds that induce oxidative reactions. Hence, the evolution of  $\text{CO}_2$  from the peat is to be charged chiefly to decarboxylation, i.e., the splitting off of  $\text{CO}_2$  from uronic acid complexes, as demonstrated by Shorey and Martin (8).

#### CHOICE OF ANTIOXIDANT

In experiments conducted in 1930, one of the writers (7) concluded that stannous chloride is the preferred antioxidant. Because of scarcity of tin, a more detailed consideration has been given the relative merits of ferrous and stannous salts in the repression of  $\text{CO}_2$  evolution from soil organic



TABLE 1.—*Relative effectiveness of ferrous and stannous salts in repression of organic CO<sub>2</sub> evolution from digestions of soils with dilute HCl*

SOIL TYPE AND CHEMICAL CHARACTERISTICS	DIGESTION PERIOD	CO <sub>2</sub> PER CHARGE,* WITH		
		O	FeSO <sub>4</sub>	SnCl <sub>2</sub>
	minutes	gram	gram	gram
Hartsells fine sandy loam } organic matter—low } Fe and Mn —low }	2	.0026	.0116	.0018
Clarksville silt loam } organic matter—low } Fe and Mn —low }	2	.0065	.0020	.0014
Bolton silt loam } organic matter—low } Fe and Mn —high }	2	.0156	.0017 .0019	.0011 .0012
		Av. .0156	.0018	.0012
	5	.0176	.0030 .0031	.0017 .0021
		Av. .0176	.0031	.0019
Tellico silt loam } organic matter—low } Fe and Mn —high }	2	.0141	.0016 .0015 .0016	.0014 .0014 .0015
		Av. .0141	.0016	.0014
	5	.0187	.0030 .0030 .0030	.0015 .0016 .0017
		Av. .0187	.0030	.0016
Clay subsoil } organic matter, practically nil } Fe—high }	2	.0010	.0010	.0010
	5	.0010	.0010	.0010
Peat } organic matter—very high } Fe and Mn —low }	2	.0055	.0054 .0052	.0056 .0052
		Av. .0055	.0053	.0054
	5	.0076	.0068 .0073	.0083 .0077
		Av. .0076	.0071	.0080

\* Charges were of 20 grams, except for peat, of which 10 grams were used.

matter during digestions in dilute hydrochloric acid. The comparisons given in adjacent columns of Table 1 indicate no differences in the effectiveness of the two antioxidants on peat and subsoil. On two soils the retardative effect of 2 minute hydrochloric acid digestions with stannous

chloride was greater than that of the ferrous salt; on two other soils the differences were within experimental error. The differences were larger and more consistent when the digestion period was extended to 5 minutes. With ferrous sulfate, this extension resulted in an increase of .0014 gram of  $\text{CO}_2$  per 20 gram, whereas the increase was less than .0005 gram of  $\text{CO}_2$ , or .002 per cent, when stannous chloride was used. It may be concluded that, under carefully controlled conditions, the results obtained with the ferrous salt during a 2 minute digestion period will be only slightly higher than those obtained by the use of stannous chloride, the difference being about .003 per cent of  $\text{CO}_2$ . With a 5 minute digestion period, however, a  $\text{CO}_2$  difference of .007 per cent may develop.

With variant steam pressure, or with variable rate of steam flow because of variable resistance in the steam line, analytical values obtained with the use of stannous chloride are more consistent than are those obtained with the use of ferrous sulfate in the dilute hydrochloric acid digestion of mineral soils.

#### CHOICE OF ACID FOR DISSOLUTION OF SOIL CARBONATES

It has been established that minute quantities of hydrochloric acid are carried by the prescribed current of steam and that this contamination is eliminated by the interposition of a scrubber bulb. Under certain conditions, however, as suggested by Hildebrand and Lundell (3), it may be desirable to use perchloric acid. Therefore, the behavior of this acid in the digestion of soils was studied, especially with inclusion of antioxidants. In obtaining the results given in Table 2 for digestions with perchloric and hydrochloric acids, with ferrous sulfate and with stannous chloride, a complete purifying train containing silver sulfate was used to catch any volatilized chlorides. Apparently, neither acid is to be preferred over the other, insofar as effect upon oxidative reactions is concerned.

#### RECOVERY OF $\text{CO}_2$ FROM DOLOMITIC LIMESTONE ADDITIONS TO HARTSELLS SANDY LOAM

In the preceding investigation it was observed that a 0.25 gram charge of dolomitic limestones of 40-mesh undergoes complete dissolution during 1 minute of refluxed digestion. To provide a margin of safety, however, a 2 minute reflux digestion period was adopted to cover incidence of all carbonate materials. The 2 minute period of digestion also admits a more precise correction for  $\text{CO}_2$  evolved from an unlimed soil in experimental series. Soils containing dolomite require finer grindings and longer periods of digestion by the older procedures. No such special grinding is required by the present procedure, and the 0.5 mm. fineness prescribed by the official methods (2, 1, 2a) is adequate. Assurance that the most recalcitrant dolomites undergo complete dissolution within this brief digestion period gives to this procedure a particular value in the determination of residues from experimental incorporations of mineral carbonates.

To test the effectiveness of the proposed procedure for the recovery of added carbonates in soils, a Harsells fine sandy loam was fortified with high-grade 0.5 mm. dolomite. The soil, alone and with additive dolomite, was analyzed in quintuplet. The results, Table 3, show satisfactory precision in the determination of charges that contained 0.050 gram, and also 0.1250 gram, of the relatively coarse dolomite. Maximal deviation from the mean is only .0003 gram in .0587 gram of  $\text{CO}_2$  and the mean deviation is .0002 gram. When the  $\text{CO}_2$  value obtained for soil alone is sub-

TABLE 2.—Comparative effects of dilute perchloride and hydrochloride acids on the evolution of organic  $\text{CO}_2$  from an acidic soil

DIGESTION PERIOD	$\text{CO}_2$ EVOLUTION PER 20 GRAM CHARGE			
	IN $\text{HCl}$ WITH—		IN $\text{HClO}_4$ WITH—	
	$\text{FeSO}_4$	$\text{SnCl}_2$	$\text{FeSO}_4$	$\text{SnCl}_2$
minutes	gram	gram	gram	gram
2	.0015	.0014	.0018	.0013
2	.0016	.0014	.0019	.0012
2	.0016	.0015	.0019	.0012
2	—	—	.0018	—
2	—	—	.0020	—
Av.	.0016	.0014	.0019	.0012
5	.0030	.0015	.0030	.0020
5	.0030	.0015	.0033	.0020
5	.0031	.0017	.0031	.0020
5	—	—	.0030	—
5	—	—	.0028	—
Av.	.0030	.0016	.0030	.0020

tracted from that of soil-plus-dolomite values, the mean  $\text{CO}_2$  recoveries from both the .0500 gram and the .1250 gram additions of dolomite indicate a  $\text{CO}_2$  deficiency of .0004 gram, or a .002 per cent of the soil charge. In view of the precision registered with each series, this hardly can be attributed to inadequacy in the analysis of the dolomited soils. It is believed that a reasonable explanation lies in the nature of the determination and variability in steam passage. The pressure in the decomposition flask during the early stages of the decomposition of the dolomite charge may cause appreciable decrease in steam flow. This may account for the apparent discrepancy in the cited determinations especially when ferrous chloride was used. It is believed that the foregoing experiments on  $\text{CO}_2$  recoveries from dolomited soils have established that the proposed procedure affords speedy results of high precision and comparable in degree to the precision shown for carbonates in the absence of soil.

TABLE 3.—*CO<sub>2</sub> recoveries from analytical additions of dolomitic limestone to a Hartsells sandy loam*

COMBINATION OF CHARGE		CO <sub>2</sub> FOUND		DEVIATION FROM AVERAGE, AS OF SOIL	CaCO <sub>3</sub> EQUIVALENCE OF CO <sub>2</sub> FOUND, AS OF SOIL	
SOIL	DOLOMITE	PER CHARGE	AS OF SOIL		PER 100 GRAMS	PER ACRE
grams	gram	per cent	per cent	gram	m.e.	pounds
20	0	.0022	.011	.000		
20	0	.0022	.011	.000		
20	0	.0020	.010	— .001		
20	0	.0023	.012	.001		
20	0	.0023	.012	.001		
Av.		.0022	.011	.001	.50	500
0	.0500	.0233	.117	.000		
0	.0500	.0235	.118	.001		
0	.0500	.0234	.117	.000		
0	.0500	.0235	.118	.001		
0	.0500	.0235	.118	.001		
Av.	—	.0234	.117	.001	5.3	5,300
0	.1250	.0586	.293	.001		
0	.1250	.0589	.295	.001		
0	.1250	.0584	.292	— .002		
0	.1250	.0589	.295	.001		
0	.1250	.0589	.295	.001		
Av.	—	.05874	.294	.001	13.4	13,400
20	.0500	.0251	.126	.000		
20	.0500	.0252	.126	.000		
20	.0500	.0250	.125	— .001		
20	.0500	.0252	.126	.000		
20	.0500	.0256	.128	.002		
Av.	—	.0252	.126	.001	5.7	5,700
Av. CO <sub>2</sub> Recovery		.0230	.115		5.2	5,200
20	.1250	.0602	.301	— .002		
20	.1250	.0611	.306	.000		
20	.1250	.0609	.305	.002		
20	.1250	.0602	.301	— .002		
20	.1250	.0609	.305	.002		
Av.	—	.06066	.303	.002	13.8	13,800
Av. CO <sub>2</sub> Recovery		.05846	.0292	—	13.3	13,300

TABLE 4.—*Apparent carbonate CO<sub>2</sub> in acidic soils of wide range of physical characteristics and chemical composition as obtained by the proposed procedure*

NO.	TYPE	SOILS	ZONE	SOURCE	ACIDITY	CO <sub>2</sub> EVOLVED PER 20 GRAM CHARGE				AVERAGE CO <sub>2</sub> IN SOIL	
						1	2	3	AVERAGE	CONTENT	DEVIATION
			inches		pH	gram	gram	gram	gram	per cent	per cent
1	Apison silt loam			Tenn.	6.0	.0023	.0021	.0021	.0022	.011	.001
2	Bolton silt loam			Tenn.	6.2	.0011	.0013	.0011	.0012	.006	.001
3	Conasauga silt loam			Tenn.	6.1	.0022	.0022	.0018	.0021	.011	.001
4	Fullerton silt loam			Tenn.	5.5	.0012	.0012	.0014	.0013	.006	.001
5	Hartsells f.s. silt loam			Tenn.	5.2	.0015	.0017	.0018	.0017	.008	.001
6	Sequoia silt loam			Tenn.	5.3	.0014	.0014	—	.0014	.007	.000
7	Tellico silt loam			Tenn.	5.7	.0014	.0014	.0013	.0014	.007	.000
8	Clarksville silt loam			Tenn.	5.2	.0019	.0021	.0022	.0021	.010	.001
9	Dickson silt loam			Tenn.	5.1	.0013	.0013	.0013	.0013	.007	.000
10	Freelands silt loam			Tenn.	4.8	.0006	.0006	.0006	.0006	.003	.000
11	Colbert clay loam			Tenn.	5.4	.0012	.0014	.0013	.0013	.007	.001
12	Cumberland silt loam			Tenn.	5.6	.0042	.0042	.0042	.0042	.021	.000
13	Cumberland silt loam			Tenn.	6.1	.0018	.0015	.0016	.0016	.008	.001
14	Cumberland clay		36.48	Tenn.	5.1	.0010	.0010	.0010	.0010	.005	.000
15	Baxter silt loam		0-8	Ala.	5.0	.0013	.0013	.0013	.0013	.007	.000
16	Davidson silt loam		0-9	N.C.	6.5	.00374	.00396	.00396	.0039	.020	.001
17	Davidson silt loam		9-16	N.C.	5.8	.0045	.0045	.0040	.00433	.022	.000
18	Leonardtown silt loam		Surface	Md.	5.4	.0013	.0015	—	.0014	.007	.001
19	Miami silt loam		Surface	R.I.	4.5	.0026	.0028	—	.0027	.014	.001
20	Wooster silt loam		Surface	Ohio	5.5	.0011	.0011	—	.0011	.006	.000
21	Drummer clay loam		A	Ill.	5.4	.0022	.0024	.0024	.0023	.012	.001
22	Carrington silt loam		B	Ill.	5.5	.0012	.0012	—	.0012	.006	.000
23	Superior sandy loam		0-3	Wis.	4.5	.0128	.0124	—	.0126	.063	.001
24	Superior sandy loam		3-8	Wis.	5.5	.0006	.0006	—	.0006	.003	.000
25	Superior sandy loam		12-30	Wis.	5.3	.0063	.0059	—	.0061	.031	.001
26	Superior sandy loam		30-40	Wis.	6.3	.0006	.0006	—	.0006	.003	.000
27	Peat*			Minn.	3.7	.0066	.0052	—	.0054	.054	.002

\* A 10-gram charge was used.

### EXTENT OF CO<sub>2</sub> EVOLUTION FROM ACIDIC SOILS OF WIDE RANGE OF CHEMICAL AND PHYSICAL PROPERTIES

In the preceding experiments, in which acidic soils were digested with hydrochloric acid and antioxidants, it was shown that some CO<sub>2</sub> was evolved from noncarbonate materials. It was concluded that the larger part is to be attributed to decompositions of the decarboxylation type. To ascertain the extent of such decomposition, additional soils of variant type were subjected to 2 minute reflux digestions with hydrochloric acid and stannous chloride. The soils from within Tennessee were matched by an equal number from outside the State. Most of the Tennessee soils contained from 2 to 2.5 per cent of organic matter. Soils Nos. 23 and 27 were of exceptionally high organic matter content. The Superior Sandy Loam suffered a 40 per cent loss on ignition and the peat contained about 80 per cent of volatile matter.

The CO<sub>2</sub> evolved from the soils by the proposed procedure is given in Table 4. The mean of the evolutions of CO<sub>2</sub> from the 18 surface mineral soils is .009 per cent, whereas that from the subsoil is only .005 per cent. If the last-mentioned value be attributed solely to inorganic CO<sub>2</sub>, the evolution due to decomposition of organic matter during the analytical digestion of the surface soils is only .004 per cent. This value may be due in part to the previously-mentioned precipitation of calcium carbonate during the drying of the soil and possibly also to absorbed CO<sub>2</sub>. Such absorption would vary with types of soils, and with a specific sample, at different seasons. The soils of high organic content show a progression of approximately .015 per cent in CO<sub>2</sub> evolution per each 10 per cent increase in organic matter content.

### COMPARISON OF STEAM DISTILLATION PROCEDURE WITH SCHOLLENBERGER METHOD

The primary objective of this comparison was to determine the extent to which the decomposition of organic matter is induced by the two procedures. Some of the soils of Table 4 were selected because of their high organic content and some because of their abnormal evolutions of CO<sub>2</sub> in previous digestions.

Table 5 shows that higher results were obtained invariably by the steam distillation procedure; the differences ranged from .0020 to .0102 gram of CO<sub>2</sub> per 20 grams of soil. The CO<sub>2</sub> evolved by steam distillation of the mineral soils was approximately .010 per cent greater than that evolved by the vacuum distillation, and about .050 per cent greater for the peat and the A<sub>o</sub> horizon of the podsol soil. In some instances the Schollenberger procedure gave increased evolution of CO<sub>2</sub> with increase in organic matter content. It is apparent, however, that such CO<sub>2</sub> evolution is governed by the nature of the organic matter, as well as by its quantity.

The results by the Schollenberger method are given in the last column

of Table 5. They indicate that the organic matter in the B horizon of the podsol soil is the most readily attacked by the dilute hydrochloric acid, whereas that in the peat is the most resistant. The distinction as to nature of organic matter is emphasized in the correlation of the results by the two procedures, as expressed by the CO<sub>2</sub> ratios for these soils in the next to the last column. Decided departure from the 1:5 ratio of CO<sub>2</sub> evolutions by the two procedures indicates that a part of the evolved CO<sub>2</sub> was of inorganic origin. Alexander and Byers (1) reported .023 per cent of CO<sub>2</sub> in the B horizon of the Davidson soil from the use of 5 per cent hydro-

TABLE 5.—Comparison of carbonate CO<sub>2</sub> values as obtained by steam distillation and Schollenberger procedures

MATERIAL USED				CO <sub>2</sub> EVOLVED PER CHARGE			CO <sub>2</sub> IN SAMPLE			CO <sub>2</sub> IN RELA- TION TO O.M.	
TYPE		ORGANIC MATTER	pH	CHARGE	BY STEAM DISTILLA- TION	BY VACUUM DISTILLA- TION	DIFF.	STEAM	VACUUM	RATIO V:S	
		<i>per cent</i>		<i>grams</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>per cent</i>	<i>per cent</i>		<i>per cent</i>
Peat		70	3.7	10	.0054	.0009	.0045	.054	.009	1:6	.013
Superior sandy loam	A <sub>1</sub>	38	4.5	20	.0126	.0024	.0102	.063	.012	1:5	.032
Superior sandy loam	B	1.2	5.3	20	.0061	.0013	.0048	.031	.007	1:5	.600
Davidson clay loam	A	4.4	6.5	20	.0039	.0022	.0017	.020	.011	1:2	
Davidson clay loam	B <sub>1</sub>	1.3	5.8	20	.0043	.0023	.0020	.022	.012	1:2	
Miami sandy loam		—	4.5	20	.0027	.0004	.0023	.014	.002	1:7	
Drummer clay loam		—	5.4	20	.0023	.0002	.0021	.012	.001	—	
Cumberland clay <sup>a</sup>		—	—	20	.0010	.0008	.0002	.005	.004	—	

\* From 36–48 inches.

chloric acid at room temperature. The result by steam distillation is in close agreement with their finding, but is .012 per cent above that by the vacuum method.

From the foregoing data and discussion, it is concluded that the CO<sub>2</sub> evolved from acidic soils by digestion with dilute solution of hydrochloric acid containing an antioxidant is derived from two sources—mineral carbonate and the decomposition of organic matter. The evolutions of CO<sub>2</sub> of organic source by the Schollenberger procedure are equivalent to from .001 to .01 per cent of the soil. Such evolutions by the steam digestion procedure are about five times as great, although the total usually is less than .010 per cent of the soil.

The extent of disintegration of organic matter during the steam digestion can be gaged by the color of the supernatant liquid. A dark brown solution indicates an organic CO<sub>2</sub> decomposition of from .03 to .05 per cent, whereas a pale yellow tint indicates an organic CO<sub>2</sub> evolution in the range .005 to .010 per cent. This estimate is precluded, however, when ferrous chloride is used as the antioxidant.

Although the extent of organic matter decomposition by steam digestion is somewhat greater than that in the Schollenberger method, such

decomposition is so small that it should present no barrier to the use of the expeditious steam distillation procedure.

### SUMMARY

A procedure for the accurate and rapid determination of soil carbonates should induce complete decomposition of carbonate, with minimal decomposition of organic matter. The demonstrated precision and rapidity of the steam distillation in the analysis of limestones prompted further study as to the adaptability of the procedure for the determination of soil carbonates and resulted in the following findings:

Dilute perchloric and hydrochloric acids can be used to equal advantage, but a scrubber bulb is essential when hydrochloric acid is used.

To minimize evolution of  $\text{CO}_2$  from decomposition of soil organic matter, it is essential to include an antioxidant, either ferrous chloride or stannous chloride, in the hydrochloric acid digestant. In general, stannous chloride proved to be the more suitable antioxidant.

Determinations on a wide range of acidic soils show that most of these soils yielded from .003 to .01 per cent of  $\text{CO}_2$  by the steam distillation procedure. Higher values were obtained from soils of the podsol type and from peat. A large fraction of this evolved  $\text{CO}_2$  is attributed to inorganic sources, since .005 per cent of  $\text{CO}_2$  was evolved during hydrochloric acid digestion of red clay virtually devoid of organic matter.

The differences between analytical results by the proposed steam digestion and those by the Schollenberger procedure are usually well within .010 per cent of  $\text{CO}_2$  for surface soils. Although not entirely eliminated, the error attributable to the decomposition of organic matter has been diminished to negligible extent. Because of the precision and rapidity attained by its use, the steam distillation procedure is proposed for the determination of soil carbonates.

### PROCEDURE FOR SOILS

*Preparation of sample.*—Grind the soil to 0.5 mm. and mix well. For soils of low carbonate content use a 20 gram charge; for those of high carbonate content, use a charge to supply ca. 0.25 gram equivalence of  $\text{CaCO}_3$ .

*Dissolvent acid.*—Dissolve 50 grams of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  per liter of dilute acid and protect by means of a solution of sodium pyrogallate.

*Apparatus and procedure.*—Use the scrubber bulb (Figure 2) for the purification of the gases; in all other respects follow the procedure prescribed for inorganic materials, Part III of this paper.

### ACKNOWLEDGMENT

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## VOLATILE OIL IN BUCHU LEAVES

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During the past 10 years analyses have been made of representative samples of many shipments of buchu leaves entering the port of New York. The yield of volatile oil and the physical and chemical characteristics of these oils were determined by the methods outlined in *Methods of Analysis, A.O.A.C.*, 1940, pp. 469-471.

Because there is a tendency to the formation of crystals in the volatile oils at room temperatures, the optical rotation was frequently made at temperatures a few degrees higher than the 20° reported in the tables. Studies have shown that in the range of 25°-40°C. variations in readings due to temperature changes are negligible. These studies extend the temperature range in regard to its effect on optical rotation as reported by Parry,<sup>1</sup> who comments as follows: "A slight correction may be made for difference in temperature, but as it has no appreciable effect on the results obtained, it may be regarded as negligible if the observation be made at any temperature between 15° and 20°C.

Buchu leaves are grown in the southern part of Africa and exported from Capetown. These are usually referred to as "Short" Buchu (*Barosma betulina*) and "Oval" Buchu (*B. crenulata*). Other varieties of buchu referred to as "Long" Buchu (*B. serratifolia* and *Epleurum serrulatum*) are known but have not been imported into New York during recent years.

Short buchu, Fig. 1B, is generally ob-ovate, light yellowish green in color, serrate edge from 12 to 18 mm. long by 6-10 mm. wide. Oval buchu, Fig. 1A, is generally oval, green on upper surface, yellowish green on the under surface crenate edge, from 12 to 20 mm. long and 5 to 8 mm wide.

<sup>1</sup> Ernest J. Parry, "The Chemistry of Essential Oils and Artificial Perfumes," Vol. 3, 3rd. ed., p. 285.

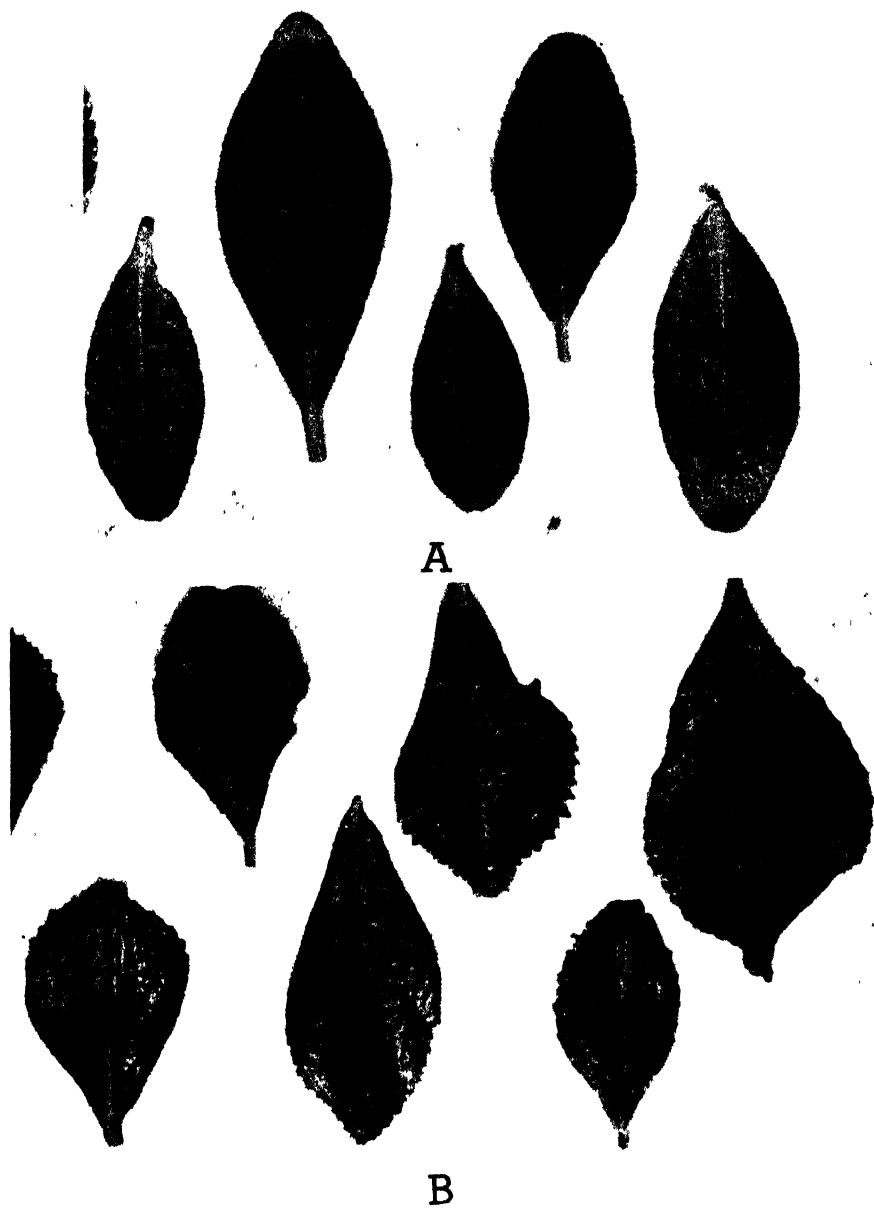


FIG. 1.-- BUCHU LEAVES, A. *Barosma crenulata*. B. *Barosma betulina*.



*Barosma Betulina* (Short Buchu)

YIELD* g/w	SPECIFIC GRAVITY 25°/25°C.	OPTICAL ROTATION 25°C.	REFRACTIVE INDEX 20°C.	ACID NO.	ESTER NO.
1.07	0.965	-24.2	1.485	4.8	30.6
1.1	0.964	-29.4	1.484	3.3	20.4
1.2	0.956	-30.1	1.483	6.3	30.0
1.5	0.966	-20.7	1.491	2.4	42.0
2.0	0.957	-20.7	1.485	4.2	48.3
2.2	0.955	-15.9	1.484	3.1	57.0
2.5	0.945	-20.8	1.482	4.4	40.0
1.1	0.945	-34.6	1.477	8.0	56.5
2.4	0.945	-27.7	1.481	7.3	28.6
2.9	0.943	-34.6	1.484	2.4	40.3
1.7	0.955	-29.4	1.481	5.3	43.5
1.3	0.947	-20.7	1.480	4.1	25.8

*Barosma Crenulata* (Oval Buchu)

1.6	0.926	-17.3	1.480	1.5	45.9
1.3	0.928	-19.0	1.479	2.9	20.0
1.2	0.934	-21.4	1.474	3.4	58.1
1.2	0.924	-20.7	1.487	1.3	54.2
2.0	0.924	-15.5	1.4775	1.0	49.9
1.7	0.947	-21.8	1.480	1.3	84.4
1.5	0.938	- 9.0	1.480		
1.3	0.938	-15.2	1.482		
3.0	0.942	-15.9	1.480		
3.5	0.949	- 8.3	1.480		
2.4	0.931	- 9.0	1.481		
1.5	0.956	-12.1	1.488		

\* Ml. per 100 grams of buchu.

## CONCLUSIONS

1. The yield of volatile oil is somewhat greater from the leaves of the oval buchu than from the leaves of the short buchu.
2. The specific gravity and acid number for the volatile oils of the short buchu are somewhat higher than in the case of the oval buchu.
3. The volatile oil of the short buchu gives a markedly larger minus rotation than in the case of the volatile oil of the oval buchu.

## DETERMINATION OF ADDED SALT IN FINELY GROUND FEEDS

By LUDVIG REIMERS (General Mills, Inc., San Francisco, Calif.)

The determination of added salt in dry non-pelleted mash feeds can be made rapidly and accurately by floating off the organic matter with

carbon tetrachloride and then titrating the chloride with silver nitrate solution.

*Methods of Analysis, A.O.A.C.*, does not provide a quantitative method for the determination of salt in mash feeds. "Cereal Laboratory Methods" (1941) of the American Association of Cereal Chemists supplies a method based on the work of Grattan and Potvin. (*This Journal* 23, 425). The method is complicated, involves a high blank, and is hardly suited to the determination of the usual quantities of added salt.

Salt cannot be accurately determined by the simple extraction of soluble chlorides with water because the blank is large and depends on the type of ingredients constituting the feeds. Poultry mashes, for example, normal in all respects except that no salt is included, will usually have a blank that is the equivalent of .25-.35 per cent of salt. Extraction tests on alfalfa meal, for instance, result in blanks of 1.75-2.00 per cent. Obviously the extraction method is inaccurate since the blank may be much higher than the salt content.

#### METHOD

A 5 gram sample is placed in a 100 ml. beaker and ca. 50 ml. of  $\text{CCl}_4$  is added. It is stirred very gently for ca. 1 minute to break up the lumps and allow the salt to settle and then permitted to rest quietly for an additional minute to obtain a more complete settling of the salt and to let the organic matter come to the surface, where most of it can be removed with a teaspoon and nearly all the remainder removed by pouring off the  $\text{CCl}_4$ . Care should be taken to prevent the salt from running out with the last portion. Any organic matter that may adhere to the sides of the beaker can easily be wiped out by means of a clean piece of filter paper. A second similar washing with 20 ml. of  $\text{CCl}_4$  is advisable. The residual  $\text{CCl}_4$  is then evaporated for at least a minute in the 100°-130°C. air oven.

About 25 ml. of water is added to dissolve the salt, which can then be filtered or decanted into a 250 ml. Erlenmeyer flask, followed by 2 or 3 washings with 10 ml. portions of water. To this salt solution is added ca. 0.05 gram of  $\text{K}_2\text{CrO}_4$ , and the chloride is titrated with a standard  $\text{AgNO}_3$  solution, preferably 0.1 N. Each ml. of 0.1 N  $\text{AgNO}_3$  solution is equivalent to 0.117% of salt.

#### EXPERIMENTAL DATA

Two salt-free feeds produced blanks of 0.006 and 0.009 per cent, which small corrections include the customarily anticipated blank.

Two feeds carefully prepared to contain 0.500 per cent of added salt yielded values of 0.502 and 0.508 per cent.

Another unground feed was found to contain 2.22 and 2.35 per cent of salt on two tests. Grinding on the Wiley mill gave results of 2.28 and 2.28 per cent. This is interpreted to mean that grinding does not reduce the salt to such a fine state that it will not settle with carbon tetrachloride, but does indicate that grinding is advisable to obtain a more representative sample.

Salt recoveries on ground pelleted feeds were 50, 82, 84, 80, 71, and 80 per cent on six tests—an average of 75 per cent. Apparently enough salt

is dissolved and subsequently deposited in pelleting in such a fine state of subdivision that the method is not sufficiently quantitative. If results are multiplied by the factor 1.33, the values should at least be as accurate as those obtained by the method of Grattan and Potvin.

The test can be made in less than 5 minutes.

## BIOLOGICAL VALUE OF SPECTRO VITAMIN A IN LIVER

By G. S. FRAPS and W. W. MEINKE (Agricultural Experiment Station, College Station, Texas)

Liver is recognized as an excellent source of vitamin A and also of other vitamins. Since biological methods for determining vitamin A take long periods of time and are otherwise expensive, shorter chemical methods are desirable. Fraps, Kemmerer, and Treichler (12) reported the relation between the vitamin A potency of liver extracts and their chemical composition. They saponified the livers with potassium hydroxide, extracted with ether, evaporated the ether, and dissolved the residue in cottonseed oil. At that time it was not considered practicable to preserve the vitamin A in the liver for the period of time required for the biological tests. Later experience showed that the vitamin A was preserved well for the feeding period if the liver was kept in a frozen condition. Since the saponification might affect the vitamin A potency by hydrolysis of vitamin A esters, the work was repeated with the liver instead of the saponified liver extract.

### EXPERIMENTAL

Most of the livers used were purchased at local markets and were of unknown origin, but a few were from steers that had been fed on known small quantities of carotene for 6 months or longer (10). The livers and mixtures of liver with corn meal were kept frozen except when they were being fed. Carotene was determined by colorimetric methods already described. (5). The spectro vitamin A content was determined by means of a Bausch and Lomb medium quartz spectrograph, 1,600 being used as the extinction coefficient for vitamin A. This method has been presented in detail in an earlier publication (3). A modified U.S.P. method (4) was used in determining the vitamin A potency by biological methods. Ten white rats were used in each group. Purified carotene dissolved in Wesson oil was used as a standard. The liver was fed twice a week, and the quantity given to each rat was so small that it was necessary to dilute a weighed amount of the liver with a weighed amount of white corn meal. This was done by grinding the liver with white corn meal in a mortar and passing the mixture through a fine mesh sieve to insure thorough mixing. By dilution it was possible to reduce the error of weighing since be-

tween 0.15 and 0.20 gram of the mixture was fed. This mixture was made fresh every 2 weeks. Two separate biological tests were made on many of the samples of the liver, and the data for both tests are given in the tables. The vitamin A potency of the carotene was considered to be 1.7 units per microgram, and the units due to the carotene present were subtracted before the value of the spectro-vitamin A in International units was calculated.

### RESULTS

Analyses of the beef livers are given in two groups in Table 1. One group includes those containing over 43 p.p.m. of spectro-vitamin A and the other group those containing less than 43 p.p.m.

The biological values of Group A of the beef livers ranged from 97.2

TABLE 1.—*Relation of spectro vitamin A of beef liver to International units of vitamin A*

SAMPLE	NUMBER OF RATS LIVING AT END OF TEST	SPECTRO- VITAMIN A	CAROTENE	BIOLOGICAL VALUE	SPECTRO- VITAMIN A
GROUP A		p.p.m.	p.p.m.	I.U./gram	I.U./microgram
65039	9	352.1	17.4	735.1	2.1
65039	10	352.1	17.4	740.5	2.1
65295	11	224.6	8.9	701.8	3.1
65774	6	183.9	21.8	678.2	3.7
65774	9	183.9	21.8	477.4	2.5
63360	8	151.0	20.4	530.0	3.5
63360	7	151.0	20.4	546.0	3.6
63364	7	127.9	38.2	495.3	3.9
63364	7	127.9	38.2	485.3	3.8
65772	3	120.0	5.5	345.0	2.9
65772	7	120.0	5.5	323.4	2.7
63367	10	100.2	19.4	257.7	2.6
63367	7	100.2	19.4	225.7	2.3
65297	10	97.0	4.0	286.8	3.0
63362	7	84.6	15.8	285.7	3.4
63362	7	84.6	15.8	291.7	3.4
65041	4	43.8	2.3	126.1	2.9
65041	4	43.8	2.3	97.2	2.2
Average	(18)	147.1	16.4	423.8	3.0
GROUP B					
65969	3	2.7	0.9	3.2	1.2
65969	9	2.7	0.9	2.1	0.8
65970	2	1.7	0.7	1.7	1.0
65970	2	1.7	0.7	0.7	0.4
65968	1	1.5	0.6	0.8	0.5
65968	3	1.5	0.6	0.5	0.3
Average	(6)	1.9	0.7	1.5	0.7

to 740.5 International units per gram, the spectro-vitamin A from 43.8 to 352.1 p.p.m., and the carotene from 2.3 to 38.2 p.p.m. After the carotene had been allowed for the International units of vitamin A per microgram of spectro-vitamin A ranged from 2.1 to 3.9, with an average of 3.0.

The livers of Group B were from experimental animals fed on rations low in vitamin A, and their vitamin A potency was very low, ranging from

TABLE 2.—*Relation of spectro vitamin A of pork liver to International units of vitamin A*

SAMPLE	NUMBER OF RATS LIVING AT END OF TEST	SPECTRO- VITAMIN A	CAROTENE	BIOLOGICAL VALUE	SPECTRO- VITAMIN A
		p.p.m.	p.p.m.	I.U./gram	I.U./microgram
65971	2	85.9	0.7	250.9	2.9
65971	3	85.9	0.7	234.9	2.8
65042	2	78.3	0.3	58.3	0.7
65042	5	78.3	0.3	51.1	0.7
65040	6	69.9	0.4	56.3	0.8
65040	5	69.9	0.4	48.3	0.7
65296	1	41.9	0.3	77.3	1.8
65294	2	34.9	0.2	19.3	0.6
65771	8	21.9	0.3	33.1	1.5
65771	10	21.9	0.3	30.6	1.1
65773	8	19.5	0.3	34.5	1.8
65773	10	19.5	0.3	34.5	1.8
Average	(12)	52.3	0.4	77.4	1.4
Average	(8)	—	—	—	1.2

0.5 to 3.2 International units per gram. The units of vitamin A per microgram of spectro-vitamin A ranged from 0.3 to 1.2, with an average of 0.7. In the work previously reported on liver extracts (6) a group of six livers containing 2-44 micrograms of spectro-vitamin A per gram, averaging 23.7, gave 1.5 International units of vitamin A per microgram of spectro-vitamin A. They were thus intermediate between the two groups of livers reported in this paper, with averages of 3.0 and 0.7 units per microgram.

The analyses of the pork livers are given in Table 2. These livers contained from 30.6 to 250.9 International units of vitamin A per gram, while the International units per microgram of spectro-vitamin A ranged from 0.7 to 2.9, with an average of 1.4. In the previous work the extract of pork livers did not differ appreciably from the beef livers, and the value of spectro-vitamin A in International units was higher than that found in the work here presented.

The vitamin A potency of livers can be calculated by the equation  $U = AS + 1.7C$ , in which U is the International or U.S.P. units per gram, S is the micrograms of spectro-vitamin A per gram, and C is the micro-



grams of carotene per gram. For beef livers containing more than 45 micrograms of spectro-vitamin A per gram the value of A would be 3.0. For beef liver containing from 43 to 3 micrograms per gram, A would be 1.5, and for those containing less than 3, A would be 0.7.

#### DISCUSSION OF RESULTS

The value of 3.0 International units of vitamin A per microgram of spectro vitamin A for livers high in vitamin A, secured in this work by direct tests of liver, is lower than the value of 4.5 units reported for the saponified ether extract in previous work (6). As the work here presented was done with the liver itself, the value of 3.0 is considered preferable. The value of 3.0 International units per microgram may be compared with that of Holmes and Corbet (8) of 3.3 U.S.P. units per microgram of spectro A for pure crystalline vitamin A with an extinction coefficient of 2100, which would be equal to 2.4 units for an extinction coefficient of 1600. Fraps, Kemmerer, and Meinke (5) report a value of 3.2 U.S.P. units per microgram for the spectro-vitamin A in butter, if no correction is made for pseudo vitamin A, and a value of 4.0 if correction is made. The value of 3.0 here reported is therefore consistent to some extent with the previous results.

The value of spectro-vitamin A in terms of International units ranges from 3 International units per microgram in liver containing more than 43 micrograms, to 0.7 in livers containing less than 2 micrograms. Spectro-vitamin A potency of butter (5) likewise averaged from 1.9 units per microgram in the lowest group, to 3.5 in the intermediate group, and 4.1 units for the highest groups, corrected for pseudo vitamin A. The spectro-vitamin A is measured from the absorption of light by its solutions at 328 m $\mu$ . Other compounds that absorb light of this wave length and interfere with the determination occur in cork, rubber, yeast, feeding stuffs, and butter and are otherwise widely distributed. They have for convenience been termed pseudo-vitamin A (3). Rats fed on rations containing no vitamin A have stored in their livers (12) as much as 9.9-19.4 p.p.m. of pseudo-vitamin A. Yeast, tankage, and other foods have been found to contain the compounds that permit the storage of pseudo-vitamin A in the liver of rats.

The pseudo-vitamin A would have a proportionately greater effect on the value of spectro vitamin A in livers low in vitamin A potency than on those in which it is high. The value of 0.7 U.S.P. units per microgram in livers low in vitamin A potency, compared with 3.0 micrograms per microgram of spectro-vitamin A for those high in vitamin A potency, is therefore due in large part to the presence of pseudo vitamin A.

Variations in the International units found for 1 microgram of spectro vitamin A are due in part to errors inherent in the biological method for measuring vitamin A, in part to variations in the quantities of pseudo-

vitamin A present, and perhaps to variations in the relative proportions of vitamin A alcohol and vitamin A esters present.

Moll and Reid (11) report the alcohol form of vitamin A to be about one-half as potent as the ester. To convert E at 1 per cent 1 cm. for 328 m $\mu$  in fish oil to units, these workers use 1800–1900 for the saponified oil and 3300–3700 for the untreated oil. From these data, 1 microgram of vitamin A is equal to 2.9–3.0 U.S.P. units for saponified material and 5.3 and 5.9 units for the untreated material. Grab (7) confirms the work of Moll and Reid and reports the conversion factor for the saponified sample as  $1800 \pm 400$  and for the untreated  $3400 \pm 600$ . Hume (9), however, failed to find any difference in the potency of the alcohol and the ester form of vitamin A. Baxter and Robeson (12) also give equal biological value to the palmitate, succinate, and acetate esters, and the alcohol when corrections are made for differences in molecular weight. These workers use 1780 as the extinction coefficient for crystalline vitamin A and a conversion factor of  $2460 \pm 227$ . This makes 1 microgram of vitamin A equal to  $4.30 \pm .39$  U.S.P. units.

#### SUMMARY

Spectro-vitamin A, carotene, and International units of vitamin A were determined in 24 tests on 13 samples of beef livers and 12 tests on 8 samples of pork livers. The vitamin A potency of 1 microgram of spectro-vitamin A averaged 3.0 International units for beef livers containing more than 43 micrograms per gram of spectro-vitamin A, 1.5 International units for those containing 1.5–2.7 micrograms, and 1.2 for pork liver containing 19.5–78.3 micrograms of spectro-vitamin A per gram.

The International or U.S.P. units of vitamin A in liver can be calculated by the formula,  $U = A S + 1.7 C$ , in which U is the International units per gram, S is the micrograms of spectro-vitamin A per gram, C the micrograms of carotene per gram, and A is 3.0 for beef livers containing more than 43 micrograms of spectro-vitamin A per gram, 1.5 for those containing 3–43 micrograms, and 0.7 for those containing less than 3 micrograms. The value of A is 1.2 for pork livers containing less than 80 micrograms per gram, and 3.0 for those containing more than that quantity. Livers contain compounds that do not have vitamin A activity, but they are determined as spectro-vitamin A.

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## METHODS FOR DETERMINATION OF FAT IN DOG FOOD\*

By E. D. SCHALL and M. H. THORNTON (Department of Agricultural Chemistry, Purdue Agricultural Experiment Station, Lafayette, Ind.)

The quantitative determination of fat in feeding stuffs and in food products is usually carried out by one of two methods, depending upon the nature of the material being analyzed. The first method consists of direct extraction of the sample with a solvent and is designated as official for use in grain and in feeding stuffs.<sup>1</sup> The second method involves extraction of an aqueous suspension of the sample, which has previously been hydrolyzed with hydrochloric acid. This method, developed by Hertwig<sup>2</sup> in 1923, has become an official method for the determination of fat in flour, baked cereal products, and noodles.<sup>3</sup> A modification of this method, which is used for the determination of fat in bread,<sup>4</sup> differs from the acid hydrolysis method chiefly in that the residue from the acid hydrolyzate is dried before being extracted.

The fact that prepared dog foods contain large quantities of cereals has led some analysts to use the acid hydrolysis method for the determination of fat in this type of product, the idea being that the ether extraction method gives incomplete recovery of the fat. The reason commonly given for the failure of this method is that the starch present becomes gelatinized during processing and occludes part of the fat, thus rendering it inaccessible to the solvent action of the ether. This theory is supported by the work of Grosfeld,<sup>5</sup> who reported that results obtained with ethyl ether were lower than those calculated from the ingredients in the case of egg noodles and alimentary pastes. Therefore, the higher results obtained with the acid hydrolysis method have usually been attributed to the removal of starch as an interfering factor rather than to any direct effect of the acid upon the fat itself. It is possible, however, that the procedure used in the acid hydrolysis method may bring about the extraction of some

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<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 356, 22.

<sup>2</sup> *This Journal*, **6**, 508 (1923).

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1940, 213, 11.

<sup>4</sup> *Ibid.*, 229, 65.

<sup>5</sup> *Z. Nahr. Genussm.*, **25**, 717 (1913).

non-fat material, which results in an apparent high fat content. Munsey<sup>6</sup> and Mitchell<sup>7</sup> have reported work that tends to support this possibility.

### EXPERIMENTAL

Preliminary work with the acid hydrolysis method showed that it was difficult to duplicate results unless a standard method of shaking was adopted. The instructions given in the method are not specific on this point. When the samples were shaken vigorously for a three minute period after each addition of solvent, it was found that consistent results could be obtained. This procedure was used in all work with the acid hydrolysis method.

The fat content of a sample of canned dog food obtained from the Ready Foods Co. and of each of the ingredients from which it was prepared was determined by both the ether extraction and acid hydrolysis methods.

The results of the analysis of the ingredients are shown in Table 1.

TABLE 1.—*Fat content of ingredients of canned dog food*

SAMPLE	FAT BY ACID HYDROLYSIS per cent	FAT BY ETHER EXTRACTION per cent
Steam bone meal	15.90	10.05
Meat scrap	10.89	9.62
Wheat bran	5.60	3.50
Soybean oil meal	3.32	0.84
Cracked wheat	2.88	1.82
Cracked pearled barley	2.67	1.48
Fresh fish	2.62	2.20
Corn grits	1.31	0.71
Tallow	100.00	100.00

The values obtained with the acid hydrolysis method are always higher than those given by the ether extraction method. This is true even in the case of meat and bone products in which no starch is present and in the case of vegetable products, such as wheat bran and corn grits, which have not been previously cooked. Therefore, it seemed to be certain that the acid hydrolysis method would give results that were higher than those given by the ether extraction method on any mixed feed regardless of its composition or its treatment during processing. This supposition is confirmed by the results of the analysis of the canned dog food. These results, together with the fat content calculated from analysis of ingredients, are given in Table 2.

The fat content of the dog food, calculated from the results obtained from analysis of the ingredients by the acid hydrolysis method, agrees

<sup>6</sup> *This Journal*, 19, 550 (1936).

<sup>7</sup> *Ibid.*, 16, 514 (1933).

TABLE 2.—*Comparison of calculated values for fat in canned dog food with values found by acid hydrolysis and ether extraction methods*

METHOD	CALCULATED FROM	FOUND
	INGREDIENTS per cent	per cent
Acid hydrolysis	2.23	2.32
Ether extraction	1.65	1.54

very well with the value determined by actual analysis of the dog food by this method. The same is true when the ether extraction method is used. Therefore, the processing of the dog food does not affect the recovery of fat by either method, and the higher results given by the acid hydrolysis method are due to an inherent difference in the two methods. Since the acid hydrolysis method gives results on simple unprocessed materials, such as wheat bran, which are higher than those obtained by the ether extraction method, it is probable that the acid hydrolyzate extract contains materials other than fats. This is further substantiated by the fact that a part of the dried extract is insoluble in petroleum benzin. The investigation up to this point is very similar to that already reported by Randle.<sup>8</sup>

A method was devised for calculating the true fat content of these extracts. This method necessitates the determination of the free fatty acids, the average molecular weight of the total fatty acids, and the glycerol content of the extracts. The periodic acid method of Allen, Charbonnier, and Coleman<sup>9</sup> was used for the glycerol determination, which was carried out on the fatty acid-free hydrolyzate from 1 gram samples of fat. This method was checked on samples of pure tristearin and found to give good values. The extracts contained an appreciable amount of free fatty acids, which were assumed to have existed in the sample as fat. Therefore, it was necessary to make a correction in the determined glycerol values to include the glycerol that had previously been esterified with the free fatty acids. This was done by calculating from the average molecular weight of the fatty acids the quantity of glycerol with which the determined quantity of free fatty acids would combine and adding this value to the glycerol found by analysis. This procedure was applied to extracts of the canned dog food obtained by both the ether extraction and acid hydrolysis methods. The results are shown in Table 3.

From these results (Table 3) it becomes evident that the extract of the acid hydrolyzate contained materials other than fats and that the true fat contents of both extracts were in good agreement. It should also be noted that the ether extract was almost 99 per cent true fat.

In an attempt to determine something of the nature of the non-fat material in the acid hydrolyzate extract, determinations of the unsapon-

<sup>8</sup> *This Journal*, 25, 864 (1942).

<sup>9</sup> *Ind. Eng. Chem., Anal. Ed.*, 12, 384 (1940).

TABLE 3.—*Analysis of extracts from canned dog food*

	ACID HYDROLYSIS	ETHER EXTRACTION
Average molecular weight of fatty acids	261	280
	<i>per cent</i>	<i>per cent</i>
Free fatty acids	12.0	6.88
Glycerol found	6.34	9.62
Corrected glycerol content of extracts (converting free fatty acids to fat)	7.68	10.34
Glycerol (theoretical for 100 % fat)	11.20	10.45
Fat calculated from glycerol content of extract (%)	68.5	98.8
Apparent fat content of prepared dog food (by analysis)	2.32	1.54
True fat content (apparent $\times$ actual per cent fat)	1.59	1.52

ifiable matter, furfural, and nitrogen contents of the extract were made. However, since none of these analyses indicated the presence of any considerable quantity of material the composition of the non-fat portion of the extract is unknown.

An extract of the acid hydrolyzate of a sample of baked dog food was also analyzed by the same procedure. This extract was found to contain 67 per cent true fat, which agrees very closely with the 68.5 per cent true fat found in the extract of the canned dog food. Therefore it is apparent that the type of processing does not materially affect the fat content of the extracts obtained from the acid hydrolyzate of dog foods.

### CONCLUSIONS

The fat content of each of the ingredients used in the preparation of canned dog food was determined by both the acid hydrolysis and ether extraction methods. The former method gave higher results in every case regardless of the nature of the material analyzed.

Higher results were also obtained on the dog food by the acid hydrolysis method. However, the fat content of the dog food agreed very well with that calculated from an analysis of the ingredients in the case of either the acid hydrolysis or the ether extraction method. This indicates that processing has no effect on fat recovery by either method.

On the basis of their glycerol content, the true fat present in both the ether extract and the acid hydrolyzate extract of the dog food was calculated. These values were found to be 98.8 per cent for the ether extract and 68.5 per cent for the extract of the acid hydrolyzate. Therefore, it appears that approximately 30 per cent of the extract given by the acid hydrolysis method consists of materials other than fat. If the extracts are considered on the basis of the quantity of true fat present, the results are approximately the same, regardless of the method used. However, this does not necessarily prove that all the fat present in the sample was recovered by ether extraction.

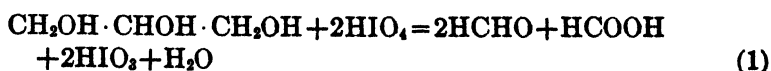
## GLYCEROL IN WINES

By MAYNARD A. AMERINE and WILLIAM C. DIETRICH\* (University of California, Davis, Calif.)

Glycerol is a normal product of alcoholic fermentation, from 7 to 14 parts usually being produced for every 100 parts (by weight) of alcohol. This determination in wine is therefore of some use in ascertaining the approximate degree of fermentation and whether alcohol or glycerol has been added. The routine determination of glycerol is usually omitted owing to the time required.

The present official method of the Association of Official Agricultural Chemists<sup>1</sup> is based on the procedure worked out by Neubauer and Borgmann<sup>2</sup> in 1878. The method gives good results for wines containing small quantities of sugar, but for wines containing 10–15 per cent of sugar, as is the case of many California dessert wines, the procedure is tedious and unsatisfactory. With sweet wines the extraction of glycerol from the gummy mass produced by the calcium hydroxide treatment is difficult and subject to manipulative errors and the recommended drying procedure is not exact.

This paper presents methods for the isolation of glycerol and its determination by periodic acid oxidation based on the general reaction of Malaprade<sup>3</sup> (*see also* Fleury and Fatome).<sup>4</sup> Polyhydric alcohols such as glycerol in the presence of dilute sulfuric acid are oxidized by periodic acid to formaldehyde and formic acid:



This reaction has been applied to the determination of glycerol in dry or nearly dry French wines by Fatome.<sup>5</sup>

The substances present in wines that react with periodic acid, besides polyhydric alcohols, are tartaric, lactic, and small quantities of other organic acids, and the hexose sugars, dextrose and levulose. The simple alcohols and malic and citric acids do not interfere. The organic acids can be precipitated with lead acetate and the sugars removed by barium hydroxide in alcoholic solution. Ferré and Michel<sup>6</sup> reported small quantities of volatile substances in wines, which reduce periodic acid. 2,3-Butylene glycol will react with periodic acid and is not removed by the procedures indicated herein. It is present only in small quantities. Mannite is found in poorly fermented wines. It may be removed by the use

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<sup>1</sup> *Methods of Analysis, A.O.A.C.*, 1940, 164.

<sup>2</sup> *Z. anal. Chem.*, 17, 442–451 (1878).

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<sup>4</sup> *Ann. fermentation*, 1, 285–290 (1935).

<sup>5</sup> *Ibid.*, 291–297.

<sup>6</sup> *Bull. inter. Vin*, 11, 37–44 (1938).

of a 3:2 ether-alcohol mixture, in which it is not soluble. If present in only small quantities, its interference is not serious.

### EXPERIMENTAL

The clarification of the wine with barium hydroxide or with lead acetate plus barium hydroxide was tested. Results are given in Table 1.

TABLE 1.—Comparison of lead acetate plus  $\text{Ba}(\text{OH})_2$  and  $\text{Ba}(\text{OH})_2$  clarification for wines<sup>a</sup>

ALCOHOL	EXTRACT	GLYCEROL		
		$\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 + \text{Ba}(\text{OH})_2^b$	$\text{Ba}(\text{OH})_2^b$	$\text{Ba}(\text{OH})_2$ (corrected) <sup>c</sup>
per cent	per cent	per cent	per cent	per cent
12.7	3.2	1.04	0.85	0.93
12.5	1.8	0.81	0.79	0.87
12.3	1.9	0.92	0.89	0.98
12.1	5.2	1.08	0.94	1.04
11.6	2.3	0.83	0.65	0.72
12.9	4.3	0.87	0.76	0.83
14.1	2.3	0.87	0.83	0.91
13.1	2.6	1.18	0.98	1.08
12.1	2.2	1.06	0.99	1.09
Average		0.96	0.85	0.94

<sup>a</sup> For the lead acetate clearing 10 ml. of wine, 2 ml. of 20% lead acetate, and 2 ml. of 40%  $\text{Na}_2\text{CO}_3$  were placed in a 100 ml. volumetric flask and brought to volume; 20 ml. of the clear supernatant liquid was placed on 2.5 grams of  $\text{Ba}(\text{OH})_2$ , left at 32° F. for 2 hours, and then made to 100 ml. with 95% alcohol. This mixture was allowed to stand 15 hours, and a 50 ml. aliquot was used for periodic acid oxidation. For the  $\text{Ba}(\text{OH})_2$  clearing 2 ml. of wine was placed on 2.5 grams of  $\text{Ba}(\text{OH})_2$  and left at 32° F. for 2 hours and then made to 50 ml. with 95% alcohol. The sample was filtered, and a 25 ml. aliquot was taken for the periodic acid oxidation.

<sup>b</sup> The use of pure glycerol (10.0 mg./ml. of solution) in the lead acetate and  $\text{Ba}(\text{OH})_2$  clarification gave 10.1 mg. of glycerol while the  $\text{Ba}(\text{OH})_2$  alone gave 9.1.

<sup>c</sup> Assuming a 91% recovery for pure glycerol with the  $\text{Ba}(\text{OH})_2$  clarification.

Clarification of wine with lead acetate and barium hydroxide gave a fair recovery of added glycerol and is recommended. Barium hydroxide clarification alone gave a low recovery of glycerol. Fleury and Fatome<sup>4</sup> obtained good recovery, but Fatome<sup>5</sup> found low results with barium hydroxide clearing alone for red wines. However, he recommends it for white wines. By using both barium hydroxide and lead acetate fairly satisfactory recovery of glycerol was obtained from solutions containing 5–15 per cent dextrose (see Table 2). With solutions of dextrose (5–15 per cent) and glycerol (0.5–1.0 per cent), erratic recovery of glycerol was obtained by the A.O.A.C. procedure. Typical data are given in Table 3.

The particular difficulties of clarifying sweet wines and of extracting glycerol from the clarified solution in the official method are recognized in a recent paper by Pritzber.<sup>7</sup> He recommends a sand-calcium oxide-magnesium carbonate procedure to insure the destruction of the sugars and to facilitate the extraction of glycerol with the ether-alcohol mixture.

<sup>7</sup> *Mitt. Lebensmitt. Hyg.*, 31, 223–229 (1940).



TABLE 2.—*Recovery of glycerol from solutions containing large quantities of sugar by the periodic procedure*

GLYCEROL ADDED	SUGAR	GLYCEROL FOUND	RECOVERY
<i>gram/100 ml.</i>	<i>grams/100 ml.</i>	<i>grams/100 ml.</i>	<i>per cent</i>
1.00	5.0	1.02	102
1.00	5.0	0.95	95
1.00	5.0	1.03	103
0.48	5.0	0.45	94
0.51	5.0	0.50	98
1.00	10.0	1.11	111 <sup>a</sup>
0.48	10.0	0.48	100
0.48	10.0	0.47	98
0.50	11.0	0.51	102 <sup>b</sup>
0.50	11.0	0.50	100 <sup>b</sup>
0.50	13.0	0.48	96
0.50	13.0	0.49	98
0.50	13.0	0.46	92 <sup>c</sup>
0.50	13.0	0.46	92 <sup>c</sup>
0.48	15.0	0.51	106 <sup>d</sup>
0.48	15.0	0.49	102 <sup>d</sup>
0.52	15.0	0.51	98 <sup>d</sup>
0.97	15.0	0.94	97
0.97	15.0	0.90	93

<sup>a</sup> Solution reclarified without using talc.<sup>b</sup> Solution reclarified using talc.<sup>c</sup> Solution reclarified with Ba(OH)<sub>2</sub> without previous lead acetate clarification.<sup>d</sup> Solution cleared with 3.75 grams of Ba(OH)<sub>2</sub>.TABLE 3.—*Recovery of glycerol from solution containing large quantities of dextrose by the official A.O.A.C. method<sup>a</sup>*

GLYCEROL	DEXTROSE	GLYCEROL FOUND	RECOVERY
<i>gram/100 ml.</i>	<i>grams/100 ml.</i>	<i>grams/100 ml.</i>	<i>per cent</i>
1.00	5.0	1.17	117
1.00	5.0	0.858	86
0.997	5.0	0.988	99
0.503	5.0	0.583	116
0.50	5.0	0.571	114
0.50	5.0	0.509	102
1.00	10.0	1.068	107
0.50	10.0	0.646	130
0.50	10.0	0.400	80

<sup>a</sup> The official method requires a double clarification for wines containing over 5 grams/100 ml. of extract, or roughly for wines containing more than 3% sugar. The procedure is to add 'successive small portions of milk of lime until wine becomes first darker and then lighter in color.' After making to volume and filtering the regular clarification is carried out. It is not clear whether 4-5 ml. of milk of lime should be added for each gram of extract in this second step or whether 4-5 ml. is added in the first and second steps together. The quantity of CaO added markedly influences the per cent recovery of glycerol. Very high results are obtained if too much CaO is added. Furthermore, the change in color from dark to light does not occur with some dessert wines and too much CaO will be invariably added in such cases.

Rothenfusser<sup>3</sup> found sugars to be fairly insoluble in absolute methyl alcohol and clarification of high sugar solutions with methyl alcohol-barium hydroxide mixture was attempted. Using various ratios of sugar to barium hydroxide, the writers found no constant factor for complete destruction of sugars concomitant with complete recovery of glycerol.

#### METHOD

##### REAGENTS

*Standard arsenious acid solution.*—0.1 N. Dissolve 4.95 grams of the sublimed oxide and 15 grams of  $\text{Na}_2\text{CO}_3$  by warming with 150 ml. of water, transfer the solution to a liter flask, add 25 ml. of 1 N HCl acid, and dilute at 20°C. to volume.

*Periodic acid solution.*—2.3 grams of  $\text{HIO}_4 \cdot 2\text{H}_2\text{O}$  in 100 ml. of water.

##### DETERMINATION

(a) *Clarification of Dry Wines.*—Place 25 ml. of wine in a 100 ml. volumetric flask. Add 1 ml. of 20% lead acetate for each 5 ml. of wine and shake; bring to volume, shake, and centrifuge or allow to settle clear. Place 2.5 grams of finely powdered anhydrous  $\text{Ba}(\text{OH})_2$  in a 100 ml. volumetric flask. Transfer into the flask 20 ml. of the lead-cleared wine, taking care not to include any of the lead precipitate. Place the tip of the 20 ml. pipet deep in the flask so that no liquid remains on the neck. Swirl the flask to break up the lumps of  $\text{Ba}(\text{OH})_2$  but avoid shaking any of the solution into the neck of the flask. Make up to volume with 95% alcohol and shake until a precipitate forms. Allow to stand for 15 hours at 0°C. Prepare a filter pad in a Büchner funnel by mixing talc and alcohol and placing a smooth layer on the filter, drying by suction. Bring to original volume by warming and filter on the Büchner funnel (or centrifuge). A thin white flocculent precipitate forming in the filtrate may be ignored. Proceed as directed in (c) or (d).

(b) *Clarification of Sweet Wines.*—If the wine contains more than 5% sugar, clarify 50 ml. with 5 ml. of 20% lead acetate in a 100 ml. volumetric flask and dilute with water to volume. Shake mixture and let settle for 1 hour. Place 10 ml. of the clear supernatant liquid on a mixture of 0.5 gram of talc and 2.5 grams of dry  $\text{Ba}(\text{OH})_2$  in a 40 ml. evaporating dish. (If sugar content is over 10% use 3.75 grams of  $\text{Ba}(\text{OH})_2$ ). Place the dish in a water bath at 50°C. for 30 minutes. Rub off any crust that may have formed on the side of the dish while still warm. Dissolve the glycerol in 50 ml. of ethyl alcohol containing 0.5 gram of talc/100 ml. Stir the mixture and keep it smooth. Filter on a Büchner funnel prepared as directed in (a). Wash with ethyl alcohol saturated with  $\text{Ba}(\text{OH})_2$ . Transfer to a 100 ml. volumetric flask and bring to volume with alcohol. Shake and let stand. Proceed as directed in (c) or (d).

(c) *Clarification with Removal of Mannite.*—Follow one of the procedures outlined previously to the stage where the sugar is removed, then pipet an aliquot of the filtrate containing 5–20 mg. of glycerol into a 50 ml. evaporating dish, add 0.01–0.1 gram of  $\text{Na}_2\text{SO}_4$  and make slightly acid with  $\text{H}_2\text{SO}_4$  (20%). Evaporate to a sirupy consistency on a water bath at a temperature not exceeding 90°C. Add 5 ml. of absolute alcohol to dissolve this residue and transfer to a 40 ml. centrifuge tube, washing the dish with successive small portions of absolute alcohol until the volume of the solution is 10 ml. Add 3 portions of 5 ml. each of anhydrous ether, shaking thoroughly after each addition. Centrifuge, decant into 125 ml. Erlenmeyer flasks, and wash the precipitate three times with 10 ml. portions of a mixture of 2 volumes of

<sup>3</sup> Z. Untersuch. Lebensm., 66, 182–192 (1933).

absolute alcohol and 3 volumes of anhydrous ether. If no centrifuge is available, pour off through a filter and wash with an alcohol-ether mixture. Add 25 ml. of water and evaporate off the ether and alcohol on a water bath. Proceed as directed in (d), starting with "Add, very accurately, 5 ml. of periodic acid. . . ."

TABLE 4.—Duplicate glycerol analysis on wines by the periodic procedure

TYPE	ALCOHOL	EXTRACT	GLYCEROL		ALCOHOL <sup>a</sup> GLYCEROL
			FIRST	SECOND	
	volume per cent	per cent	per cent	per cent	
Angelica	19.4	13.6	1.14	1.19	—
Angelica	20.8	12.9	0.64	—	—
Burgundy	12.6	2.5	1.12	1.13	8.88
Burgundy	13.6	2.9	1.10	1.06	9.98
Cabernet	11.1	2.7	0.91	0.94	9.53
Cabernet	11.6	2.9	1.02	1.04	8.95
Muscatel	19.8	12.9	0.55	—	—
Muscatel	19.4	14.4	0.51	—	—
Port	19.3	12.0	0.55	0.53	—
Port	19.5	11.1	0.60	0.58	—
Riesling	12.2	2.1	1.01	0.98	9.73
Riesling	11.4	2.0	0.84	0.85	10.71
Sauterne (dry)	12.3	2.1	0.93	0.91	10.62
Sauterne (dry)	12.2	2.9	1.06	0.98	9.49
Sherry (sweet)	20.0	5.7	0.95	0.93	—
Sherry (sweet)	19.7	5.2	1.14	1.15	—
Sherry (dry)	19.0	3.5	1.10	1.11	—
Sherry (dry)	18.9	4.3	0.99	0.90	—

<sup>a</sup> The alcohol/glycerol ratio of the table wines was calculated from the alcohol and glycerol expressed as grams/100 ml. The alcohol/glycerol ratio of the dessert wines (Angelica, muscatel, port, and sherry) is affected by fortification and is omitted here.

(d) *Determination of Glycerol*.—Pipet an aliquot of the filtrate containing 5–20 mg. of glycerol (usually 25 ml.) into a 125 ml. Erlenmeyer flask. Add 25 ml. of water and 0.01–0.1 gram of  $\text{Na}_2\text{SO}_4$ . Add 1 drop of phenolphthalein and neutralize with 2 *N*  $\text{H}_2\text{SO}_4$ , adding 4 drops in excess. Place the flask on a steam bath and evaporate to ca. 20 ml. to remove most of the alcohol. Do not heat the sample higher than 90°C. Add, very accurately, 5 ml. of periodic acid to the flask. Wash down the sides of the flask with water and let stand 15 minutes. Add ca. 5 ml. of saturated  $\text{Na}_2\text{CO}_3$  to bring to a faint pink color of phenolphthalein (neutral or slightly alkaline). Add 5 ml. of ca. 1 *N* KI solution, and using starch as indicator titrate the liberated iodine with 0.1 *N* arsenious acid. The number of ml. used is (A). Determine

the ml. of arsenious acid (B) equivalent to 5 ml. of the periodic acid. Mg. glycerol =  $(A - B) \times 4.6/2$ .\*

### DISCUSSION AND RESULTS

The results of duplicate analysis on types of California table and dessert wines are given in Table 4. The per cent glycerol in several types of California table wines varied from 0.84 to 1.12 per cent and in dessert wine types from 0.51 to 1.14 per cent. The alcohol/glycerol ratio was fairly constant except in one Angelica sample, which may have had glycerol added. The method of calculating the alcohol/glycerol ratio in dessert wines and the results obtained will be discussed further in a later publication.

### SUMMARY

The periodic method of Fleury and Fatome for the determination of glycerol in wines has been examined in regard to its applicability to California wines, with particular reference to the sweet types. There is good elimination of sugars with lead acetate-barium hydroxide clarification. A method for the periodic acid oxidation of glycerol in the clarified solution is outlined.

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## ACIDITY IN ALCOHOLIC BEVERAGES

By G. B. LEVY and M. ROSENBLATT (Schenley Research Institute, Inc., Lawrenceburg, Ind.)

The acid content of distilled liquors such as whiskey and brandy is usually considered an important criterion for evaluation of quality and age.

The difficulties encountered in the determination of the acid content of distilled liquors are generally recognized (1), but it appeared to be desirable at this time to make a complete study of the factors, individual and composite, affecting this determination. In this paper difficulties caused by the nature of materials analyzed and those caused by the technic of analysis are distinguished.

The factors involved in these determinations are: (1) Nature of acids, (2) influence of alcoholic content, (3) influence of dilution, (4) method of preparation of samples, (5) effect of indicators, and (6) effect of color of samples.

Throughout these investigations, a uniform technic was used. In order to eliminate potential sources of errors, or to keep them constant within known limits, all titrations were carried out with the aid of the glass electrode, the exclusion of  $\text{CO}_2$  and at constant temperature.

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\* The residual periodic acid may also be measured by adding excess arsenious acid and titrating the excess with 0.1 *N* iodine in neutral solution.

## DESCRIPTION OF APPARATUS

The titration vessel (Figure 1) was constructed from an inverted, round, 500 ml. Pyrex bottle, the bottom of which was cut off. A rubber stopper was sealed into the neck of the bottle. Through a hole in the stopper there

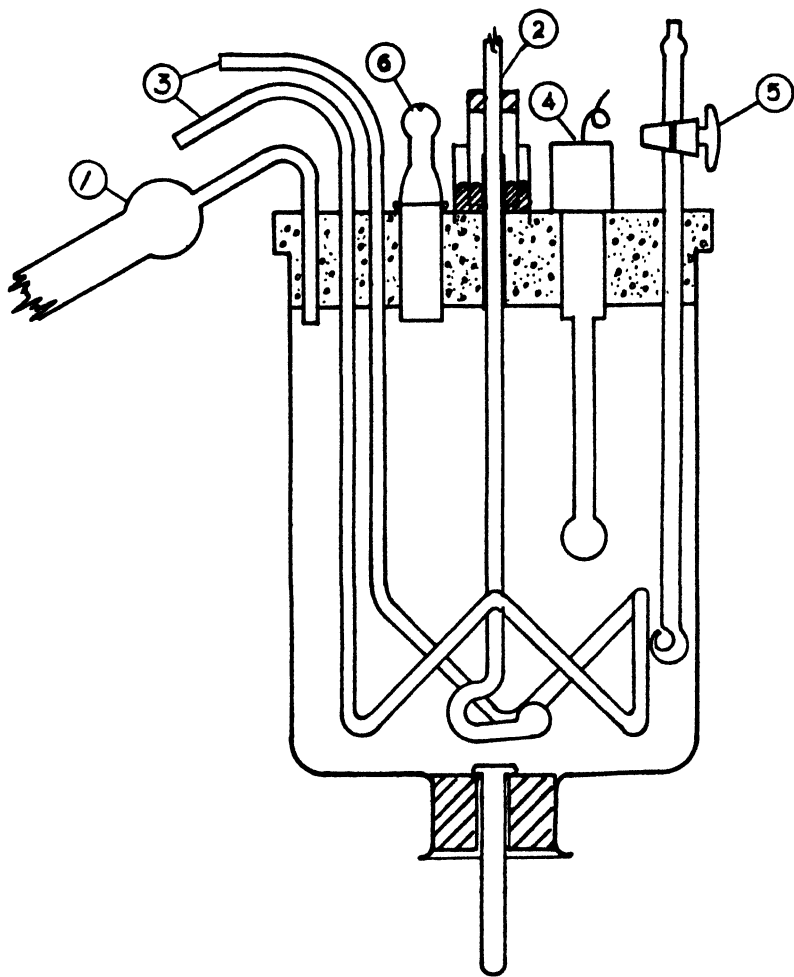


FIG. 1.—POTENTIOMETRIC TITRATION ASSEMBLY.

was inserted a glass rod, the diameter of which was slightly smaller than the hole. This rod had a flattened end, which acted as a valve. The top of the titration vessel was closed by a paraffined cork stopper, which was covered with sealing wax. This stopper had several openings through which the following objects were inserted:

- (1) Sodium-calcium-hydrate absorption tube.
- (2) Stirrer with mercury trap.

- (3) Intake and outlet for cooling (heating) water of 25°C. ( $\pm 0.2$ ), which was syphoned from a thermostat.
- (4) Glass electrode (Coleman 3001F).
- (5) Glass hook containing saturated KCl solution (Coleman 3014A), which was connected with a KCl reservoir by a rubber tube. This reservoir held a calomel reference electrode (Coleman 3001A). Both this electrode and the glass electrode were in electric contact with a Coleman pH electrometer (Model 3D).
- (6) A short tube ca. 10 mm. in diameter, onto which a rubber baby-bottle nipple was fitted. Into this nipple a slot was carefully cut in the form of the letter V. Thus it was possible to push pipets, funnels, or a buret through the nipple for introducing the reagents into the vessel. When these objects were withdrawn, the tonglike section created by the V-shaped cut sprang back and closed the nipple and the container.

The titration vessel had a capacity of about 300 ml., and 200–275 ml. of liquid was used for each titration. In order that the pH values measured should represent the true values (as defined by the hydrogen electrode) certain precautions were necessary.

It had been found previously by Liebmann and Rosenblatt (2) that the glass electrode is suited for pH work with whiskey; and Dole (3) gives the error due to the ethyl alcohol content as 0.08 pH unit at 55 per cent ethyl alcohol and only 0.05 pH unit at 33 per cent ethyl alcohol. As the accuracy of the measurements is about 0.05 pH units no corrections were made for the ethyl alcohol content, but care was taken never to have liquids with more than 60 per cent ethyl alcohol content or with a pH value above 10.5 come in contact with the glass electrode. In this manner possible deterioration (temporary or permanent) of the glass electrode was prevented. The instrument was set at pH 4.01 with acid phthalate buffer and at pH 9.20 with a borax buffer. Frequent rechecks were made, and at no time was there a deviation found in excess of 0.05 pH units even after 8 or more hours.

The water used in the course of all experiments was distilled once, boiled for 15 minutes, cooled, and maintained CO<sub>2</sub>-free (sodium-calcium hydrate). The sodium hydroxide was prepared with the distilled water and maintained under similar precautions. Its strength was about 0.05 *N* (0.07 *N* in some experiments).

## RESULTS

1. *Nature of Acids.*—Thorough studies are available concerning the changes of acid content with aging (4). Somewhat expanding the range of previous investigations, the writers titrated composite Bourbon whiskey samples 0, 1, 3, and 6 months old, as well as some 1, 1½, 2, and 3 years old, for total acid content.

The results are similar to those obtained by Schiektanz and Etienne (5), i.e. with advancing age the total acid content increases and the titration curves flatten out. The greatest differences with respect to both of these

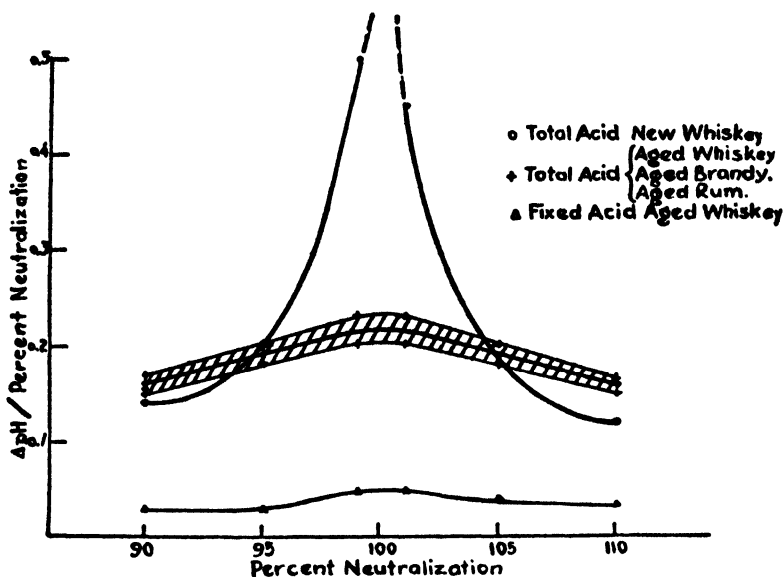


FIG. 2.—INFLECTION POINT CHARACTERISTICS DERIVED FROM POTENTIOMETRIC ACID TITRATION CURVES OF ALCOHOLIC LIQUORS.

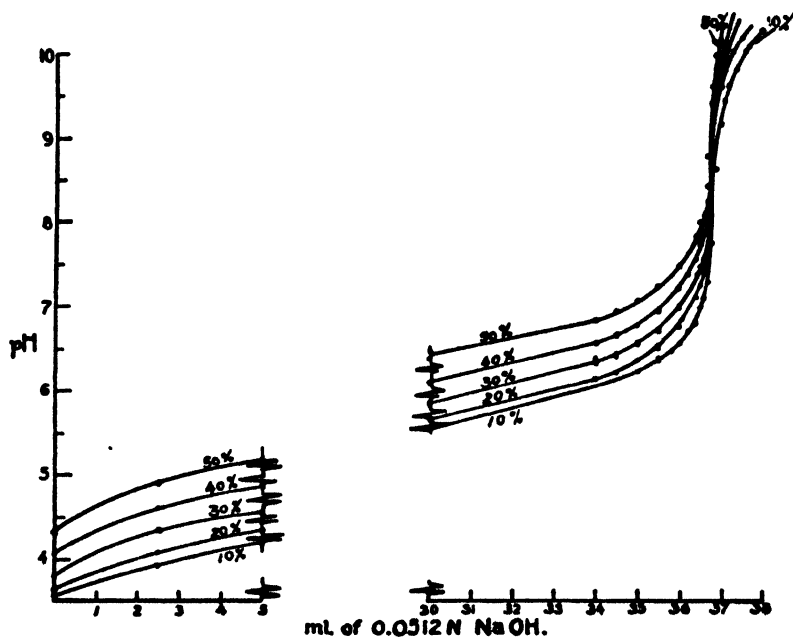


FIG. 3.—POTENTIOMETRIC ACID TITRATION CURVES OF SYNTHETIC WHISKEY SAMPLES AT VARIOUS ETHANOL CONCENTRATIONS.

features were found in products between 0 and 1 month of age. Due to similarity to previously published material, the titration curves are not presented here; however, from the titration curves the ratio of the change in pH to the per cent neutralization was determined. These ratios of various samples are plotted against the per cent neutralization in Figure 2. The 100 per cent neutralization point for each curve was chosen as the point of inflection. The shaded region includes all the points of the total acid determinations of aged whiskeys, including 7½ and 19 year samples.

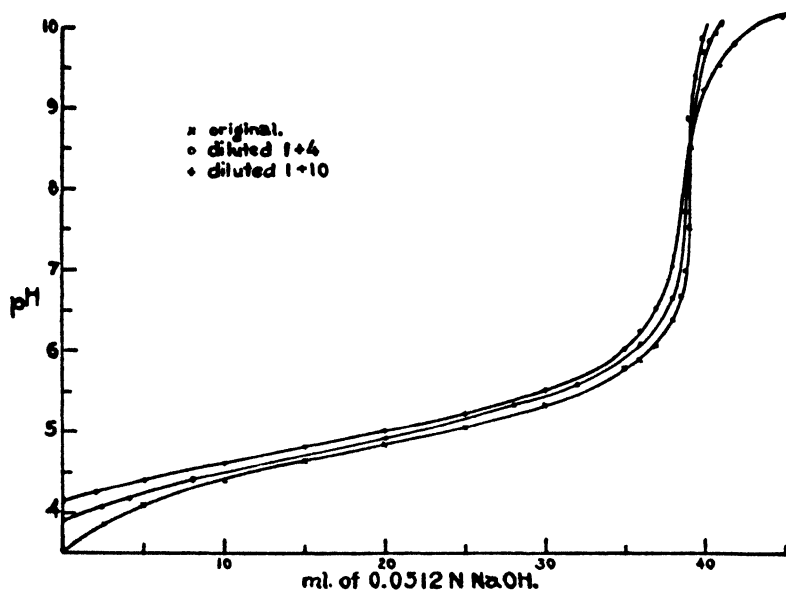


FIG. 4.—POTENTIOMETRIC ACID TITRATION CURVES OF AQUEOUS ACETIC ACID SOLUTION AT VARIOUS DILUTIONS.

The upper curve designates a zero age or "new whiskey" sample. The lower curve represents the fixed acidity determinations of an aged whiskey.

2. *Effect of Ethyl Alcohol.*—In order to study the effect of alcohol on the titration characteristics, samples containing acetic acid, water, and ethyl alcohol were so prepared that their respective alcoholic content would be 10, 20, 30, 40 and 50 per cent at neutralization. In all cases the acetic acid concentration was identical (approximately 60 grams/100 liters). The results of duplicate titrations are represented in Figure 3. The values of the titrations of the solutions containing ethyl alcohol between 0 and 10 per cent can not be shown as the curves practically coincide.

3. *Effect of Acid Concentration.*—The titration characteristics of solutions containing acid at various concentrations are well known and can



easily be calculated for individual cases. However, for the sake of completeness, three curves are included in Figure 4 to represent actual titration of an acetic acid solution and of samples of the original solution diluted 5 and 11 times. The curves in Figure 4 have been corrected for the respective dilutions in order to bring all curves into the same range.

4. *Composite Effect of Dilution.*—Obviously, in practice both effects of dilution (decrease in alcohol and acid content) will come into play. Consequently the composite effect of dilution is of greater interest than either of the single effects. Figure 5 shows titration curves of an original whiskey

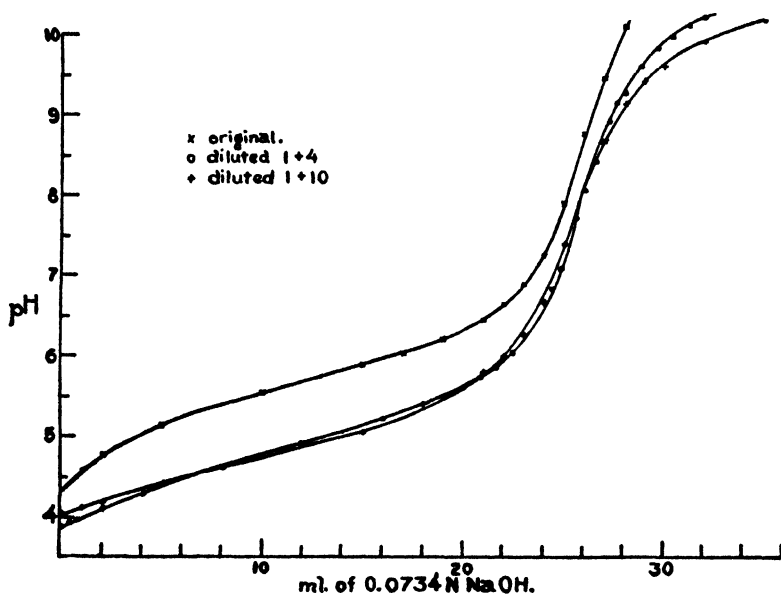


FIG. 5.—POTENTIOMETRIC ACID TITRATION CURVES OF A WHISKEY SAMPLE AT VARIOUS DILUTIONS.

sample (composite Bourbon whiskey average age 3 years) and the same sample diluted 5 and 11 fold. The curves are shown in the same range, being corrected as noted above.

5. *Preparation of Sample.*—The method of preparation of sample for the total acid determination consists merely in dilution of the sample. For the fixed acid determination, however, it is more involved. Here, the sample is first evaporated to dryness, dried at 100°C. for one hour, taken up in 50 per cent ethyl alcohol, and titrated at proper dilution (6). The official A.O.A.C. method defines the total and fixed acidity fractions of an aged whiskey and presents a definite analytical technic for the determination as described above. Curves a and g in Figure 6 represent these fractions as potentiometric titration curves of a composite Bourbon whiskey. However, if the same whiskey sample is subjected to steam dis-

tillation, a procedure similar to that used for wine (7), a clear volatile acid fraction is obtained. The distillate and the residue were potentiometrically analyzed and are shown as curves  $b_1$  and  $b_2$  in Figure 6. The same whiskey was also subjected to vacuum distillation (30 mm.), and a similar pair of titration curves,  $c_1$  and  $c_2$ , shown in Figure 6, resulted.

In the same manner, curves  $d_1$  and  $d_2$  were obtained by another vacuum distillation (23 mm.) and plotted in Figures 6 and 7. The residue of a similar distillation was further heated under vacuum, and the resulting acidity as decreased with time is shown as curves  $e_2$  and  $f_2$  in Figure 7.

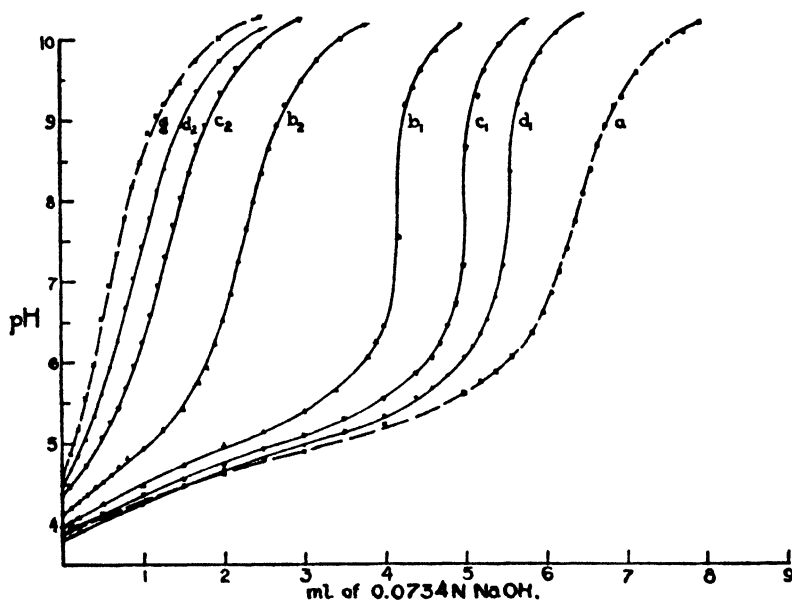


FIG. 6.—POTENTIOMETRIC TITRATION CURVES OF TOTAL, VOLATILE, AND FIXED ACID FRACTIONS OF A WHISKEY SAMPLE.

It is to be noted that the volatile fraction remained constant as indicated by the colinear points  $d_1$ ,  $e_1$ , and  $f_1$  in Figure 7. Curve  $h$  in Figure 7 is the potentiometric titration of the residue of the same Bourbon whiskey after evaporation on a water bath and omission of subsequent heating.

6. *Effect of Indicators.*—Since the internal indicator technic is in general use for the titrations under study, the influence of added indicators was investigated. In general laboratory practice, the concentration of indicators in titrations is usually negligible as compared to the other substances present. This, however, is not so in the case under discussion. When 2 ml. of 1 per cent phenolphthalein solution is added to the acid solution a large quantity of weak acid is also added.

The acid analyzed is 0.1–0.5 millimol, whereas the quantity of phenolphthalein itself is over 0.06 millimol. The effects of the addition can be

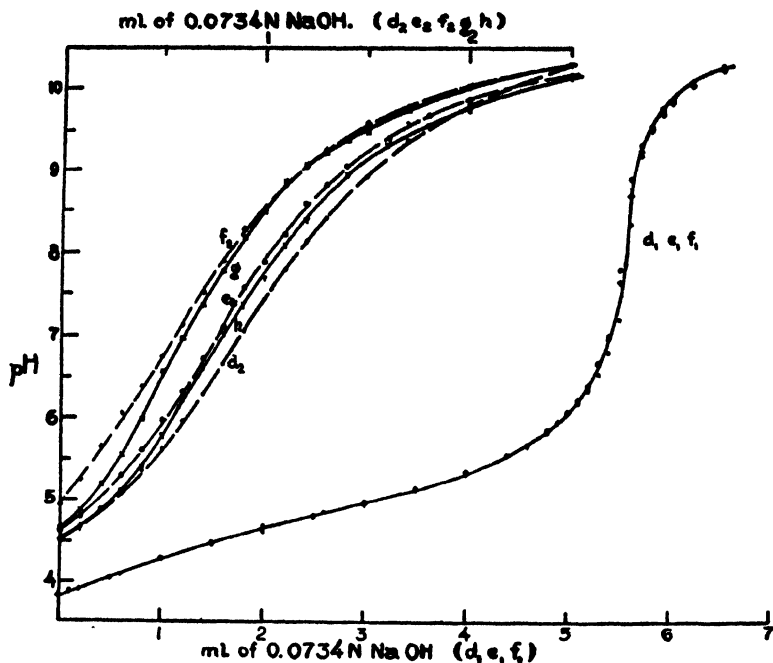


FIG. 7.—POTENTIOMETRIC TITRATION CURVES OF VOLATILE AND FIXED ACID FRACTIONS OF A WHISKEY SAMPLE.

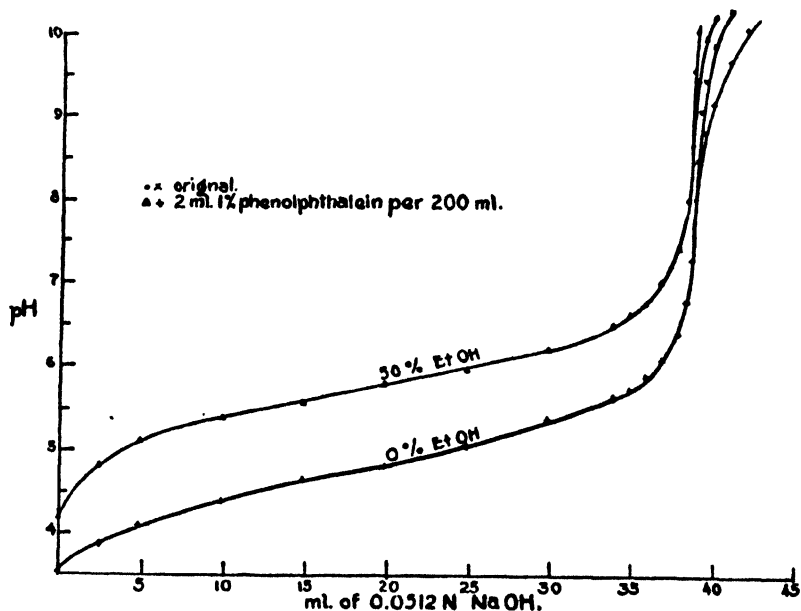


FIG. 8.—EFFECT OF PHENOLPHTHALEIN INDICATOR ON THE POTENTIOMETRIC ACID TITRATION CURVE OF A SYNTHETIC WHISKEY SAMPLE.

seen in Figure 8. The curves represent titrations of synthetic whiskey samples (60 grams acetic acid/100 liters) of 0 and 50 per cent alcohol content, with and without the addition of 2 ml. of 1 per cent phenolphthalein solution for 250 ml. of solution.

Figure 9 shows titration curves of a composite whiskey sample—the original sample; with 0.4 ml. of 1 per cent  $\alpha$ -naphtholphthalein added; and with 2 ml. of the same indicator solution added.

7. *Effect of Color of Samples.*—The color of the samples of aged distilled liquors is a dark brown. Even when diluted according to accepted practice

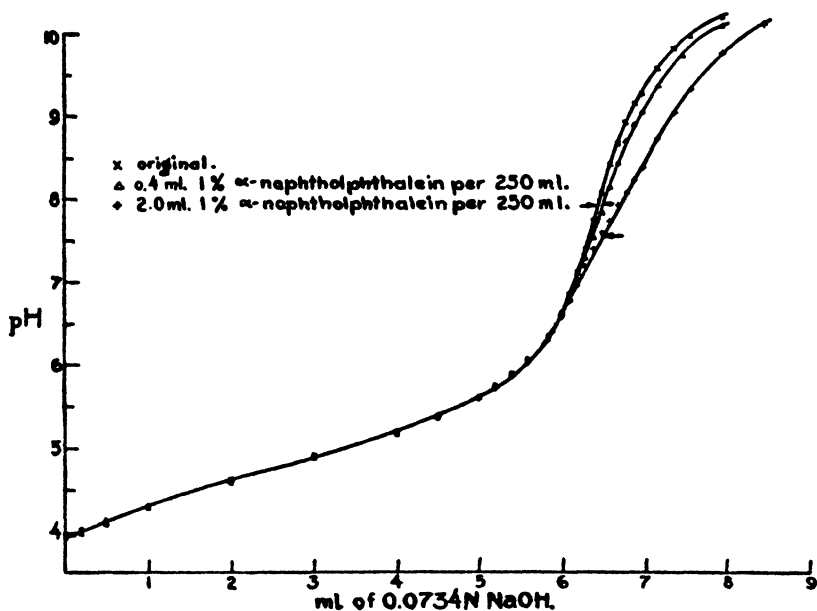


FIG. 9.—EFFECT OF  $\alpha$ -NAPHTHOLPHTHALEIN INDICATOR ON THE POTENTIOMETRIC ACID TITRATION CURVE OF A WHISKEY SAMPLE.

it is yellow to deep yellow. It is well known that this color deepens considerably and changes into an orange-brownish hue near neutral point. This is caused by the indicator-like substances present in the aged liquors.

It is apparent that in order to detect the pH with the aid of an indicator it is necessary to add large quantities. This is especially true in the case of red indicators, the color of which blends very well with that of the sample. For example, when titrating a 6 year old Bourbon whiskey sample according to the official method the visual end point was found to correspond to pH 8.10, but when only five drops of phenolphthalein solution were added the first visible pink color appeared only at pH 8.71. A similar effect occurs with other indicators, and arrows in Figure 9 indicate the visual end point of the titration. When 0.4 ml. of  $\alpha$ -naphtholphthalein was added to the sample the end point (distinct green) was

observed at  $pH$  7.9; when 2 ml. of the same indicator solution was present the same color appeared at  $pH$  7.5.

### DISCUSSION

To base the evaluation of the methods of analysis on the foregoing experiments seems quite justified for two reasons: (1) The experiments presented were selected from a large number of titrations performed (about 150), none of which gave contradictory evidence; (2) no results thus obtained were in conflict with either pertinent publications or with general laboratory experience.

*Total Acid Content.*—All evidence points toward the conclusion that in the course of aging not only the total quantity but also the number of weak acids increases. This is confirmed by the noticeable flattening of the titration curves of samples with increasing age. It would appear that the greater the age of a sample, the more difficult it would be to titrate, with attendant loss in precision. Actually, however, the flattening of the titration curve is more or less counteracted by the absolute increase in acidity. This becomes clear when the titrations are considered on a percentage basis rather than on the conventional volume of sodium hydroxide used. Figure 2 indicates that individual differences in whiskey samples with respect to the  $\Delta pH$  per cent neutralization ratio within one age group are approximately as large as differences between various age groups, as exhibited by the narrow shaded region.

One of the important changes with aging as related to the acid determination is the deepening of color. When the analyst is working with internal indicators this represents a serious problem. The official method, prescribing dilution, counteracts this to some extent, and the dilution of samples must be considered necessary from this point of view. The effect of dilution is twofold—it decreases the alcohol concentration and at the same time the acid concentration.

The effect of the reduction of the alcohol concentration can be seen in Figure 3. With decreasing ethyl alcohol concentration the titration curves shift toward lower  $pH$  regions without significant change in shape. This effect is marked at higher levels. Between 10 and 0 per cent ethyl alcohol practically no further change occurs. The lowering of the acid concentration (Figure 4), on the other hand, does not change the  $pH$  level at all, but merely increases the flattening of the titration curve. This effect becomes noticeable only at excessive dilutions.

The composite effect (Figure 5) therefore consists of the following: At increasing dilution the titration curve first shifts towards lower values without much change in shape. When the ethyl alcohol concentration of about 10 per cent is reached (1:4–1:6 dilution) the curve becomes stabilized with respect to  $pH$  level. Increasing dilution then causes an increasing flattening, centering at the inflection point. This fact again points

to the importance of the inflection point. In general, it coincides with the neutralization point, i.e., the theoretical end point of a titration. This is not strictly true in the case of weak acids when titrated with strong base (8). However, when the concentrations are very low, such as in the case under discussion (1/100–1/1000 *N*), for all practical purposes the inflection point is identical with the end point of titration.

As pointed out previously, this inflection point (together with the whole titration curve) shifts toward lower *pH* values upon dilution of the sample. At 10 per cent ethyl alcohol concentration or less, however, the point is stable, and therefore its position with respect to *pH* can be determined.

From all data available it is apparent that the titration curves of aged liquors are flat, i.e., there is no marked inflection point but rather an inflection region. This region centers with regularity around *pH* 7.6–8.0. In the curves from which Figure 2 was drawn, the inflection points were *pH* 7.75, 7.70, 7.75, 7.67, and 7.90. Similar inflection points can be observed in other figures presented.

If the total acid content of aged distilled liquors is to be determined by potentiometric titration, as was proposed in analogous cases (9), *pH* 7.8 for a diluted sample could be taken as a representative average value for end point of titration. The electrode (preferably glass) would then act as an internal indicator. However, if a dye is to be used as internal indicator, further considerations are necessary. In order to detect a color change against the dark (orange) background (the color of the sample), it is necessary to add large quantities of indicator, which causes a considerable depression of the titration curves. Consequently, it is desirable to choose an indicator that has a contrasting color (blue or green), permitting the use of smaller amounts of same, and in order to counteract the depression of the titration curve, one that has a range lower than the end point of titration (i.e. *pH* 7.8).

Figures 8 and 9 show conclusively how the indicator (acid) depresses the titration curves as compared to the original sample.

On this basis,  $\alpha$ -naphtholphthalein was taken as an indicator. The arrows in Figure 9 indicate the observed end point (first distinct green color). It can be seen that neutralization occurred in the original sample at *pH* 7.85, corresponding to 6.45 ml. of 0.0734 *N* alkali; with the addition of 0.4 ml. of indicator the end point was observed at *pH* 7.90, corresponding to 6.50 ml.; and with the addition of 2 ml. of indicator, the end point was observed at *pH* 7.50, also corresponding to 6.50 ml. Thus a proper indicator compensates more or less for the depression of the titration curve.

*Volatile and Fixed Acidity.*—It appears from Figures 2, 6, and 7 that the titration curves of the fixed acid fractions are different in character from the total acid curves. The fixed acid curves are much flatter, having an extended inflection region from *pH* 6 to *pH* 8. The center of that region

is estimated to be about pH 7. The volatile fractions vary according to the method of preparation. The steam distilled fraction appears to consist of acetic acid with a marked inflection point around pH 8.1. The vacuum distilled fractions result in somewhat flatter titration curves, but their inflection points are in the same region.

The total acid content may be determined directly or calculated as a sum of the "fixed" and "volatile" acids. Table 1 presents a summary of the results shown in Figures 6 and 7.

Although the total acidity is constant, the first five rows in Table 1 indicate that the component fixed and volatile acidity fractions may vary considerably, depending upon the method of preparation of the sample.

TABLE 1.—*Total, fixed, and volatile acidity of aged whiskey*

PREPARATION	ML. OF NaOH		
	VOLATILE ACID	FIXED ACID	TOTAL ACID
A.O.A.C.	(5.78)*	0.62 (g)	6.40 (a)
Fixed acid by simple evaporation	(5.60)	0.80 (h)	6.40 (a)
Steam distillation	4.24 (b <sub>1</sub> )	2.14 (b <sub>2</sub> )	(6.38)
Vacuum distillation 1	5.07 (c <sub>1</sub> )	1.23 (c <sub>2</sub> )	(6.30)
Vacuum distillation 2	5.58 (d <sub>1</sub> )	0.88 (d <sub>2</sub> )	(6.46)
Vacuum distillation and drying of residue	5.58 (e <sub>1</sub> )	0.78 (e <sub>2</sub> )	(6.36)
Vacuum distillation and further drying of residue	5.58 (f <sub>1</sub> )	0.58 (f <sub>2</sub> )	(6.16)

\* Letters in parentheses refer to corresponding curves in Figures 6 and 7; numbers in parentheses are calculated.

Indeed, the A.O.A.C. method is merely one of several available arbitrary choices (10).

It appears that there is no "true" fixed and volatile acid content of an aged whiskey, and that these characteristics relate to a given method. The total acid content, however, can be considered as a true characteristic, since a direct determination and calculation under varying conditions will give identical results. In the event that the calculated total acid is less than the experimental total acid value, destruction of fixed acids may generally be assumed.

This evidence may be interpreted as follows: In whiskey or similar products, the total acidity is composed of a steam-distillable fraction, a volatile fraction that cannot be distilled with steam, and a fraction that cannot be distilled at all. The last-named fraction is slowly decomposed when heated in dry state.

Concerning the analytical features, it is clear that the determination of the volatile fractions presents no difficulty. These fractions have steep titration curves and correspondingly sharp inflection points. When an indicator (e.g., phenolphthalein) is used, a few drops are sufficient, this frac-

tion being colorless, and no disturbing indicator effects (such as those discussed previously) will be present.

The fixed acid fraction, on the other hand, is rather difficult to determine because (1) owing to the manner of preparation, part of the acidity will almost invariably be destroyed; (2) even when this destruction is avoided the flatness of the titration curve will make a determination difficult to evaluate; and (3) when indicators are used, it must be considered that this fraction contains all the coloring matter and that therefore all difficulties due to this factor are again present to a greater extent.

It appears that the total acidity can be split into two fractions, one of which—from the point of view of the analyst—contains all desirable properties, and the other all undesirable ones.

## PRECISION AND ACCURACY OF ANALYSIS

### A. PRECISION

1. *Total Acid Determination.*—The precision of the total acid determinations, when the inflection point is used as an end point, can be read from Figure 2. If it is assumed that an indicator color change can be detected within 0.2 pH units, the precision of the analysis will be about  $\pm 0.5$  per cent. If an arbitrary end point other than the inflection point is used as the end point, then the precision will suffer. This decrease in precision will be appreciable although not excessive if the end point chosen lies between pH 7.0 and 8.5.

2. *Volatile Acid Determination.*—The limits of precision in the determination of volatile acidity are determined by the buret used, normality of alkali, and similar factors, rather than by the titration characteristics of this fraction, which behaves like a new whiskey. In this case, the pH change is 0.5 or more in the range  $\pm 1$  per cent from the neutralization point. Consequently, a precision of  $\pm 0.3$  per cent or better can be obtained.

3. *Fixed Acid Determination.*—The precision of the fixed acid determination is very limited, being only about  $\pm 2$  per cent (Figure 2, lower curve). If an arbitrary end point other than the inflection point is chosen, then the precision expected will not be more than  $\pm 3$  per cent.

All the foregoing considerations were based on the assumption that in the course of the titration a definite color change will be perceptible within 0.2 pH units. Actually this is an average value rather than a constant one. In the case of newer whiskey, spirit blends, etc., this value might be somewhat less, and in the case of longer aged products or fixed acids somewhat more.

A contrasting indicator (blue or green) will have a narrower range in which perception of color change will occur than a non-contrasting one (yellow, orange or red). Also the background might modify these estimates slightly.



The above estimates represent, however, the theoretical precision, i.e., the limit of precision that can be obtained in total, volatile, and fixed acid determination by the use of an internal indicator under optimum conditions.

## B. ACCURACY OF DETERMINATION

The accuracy of the titrations in a region immediately about inflection point is the same as the precision; the analysis will be inaccurate only to the extent of the scattering of the values. When a *pH* other than the inflection is chosen for the end point, the scattering of the analysis will occur around a point other than the true value, e.g., when a higher *pH* is chosen, in addition to the scattering ( $\pm$  error), there will always be a constant (+) error. The magnitude of this constant error will depend on the *pH* chosen and on the shape of the titration curve. This shape in

TABLE 2.—*Comparison of indicators*

OBSERVER	ML. OF NaOH							
	SAMPLE A		SAMPLE B		SAMPLE C		SAMPLE D	
	$\alpha$ -NPH.	PHPTH.	$\alpha$ -NPH.	PHPTH.	$\alpha$ -NPH.	PHPTH.	$\alpha$ -NPH.	PHPTH.
1	2.46	2.62	1.77	1.90	2.63	2.80	3.32	3.52
2	2.42	2.53	1.74	1.84	2.57	2.73	3.26	3.42
3	2.44	2.61	1.77	1.79	2.58	2.91	3.22	3.48
4	2.47	2.68	1.76	1.94	2.61	2.84	3.24	3.54
Average	2.45	2.61	1.76	1.87	2.60	2.82	3.26	3.49
Standard div.	0.019	0.053	0.012	0.057	0.024	0.065	0.037	0.046
Potent. inflection pt.	2.48		1.78		2.65		3.35	

turn depends on the individual sample, the dilution, and the amount of indicator used.

Thus it is not simple to predict the exact amount of this error. Some approximate estimation, however, can be made. If *pH* 8.1 is taken as an arbitrary end point, for an average sample, 1:10 dilution and 2 ml. of phenolphthalein for each 250 ml., the error will be from  $+2\frac{1}{2}$  to  $+8$  per cent. If the same end point is taken in the fixed acid determination, the error will run  $+25$  to  $+40$  per cent.

In Table 2 the results of a small collaborative experiment are given as confirmation of the above discussion. Four observers, all well acquainted with the phenolphthalein titration (A.O.A.C.) and two of whom had never executed any titration with  $\alpha$ -naphtholphthalein, were given four different samples of whiskey. The only instructions given were to titrate in one determination according to the standard method and in the second with 0.5 ml. of  $\alpha$ -naphtholphthalein to the first distinct green color.

It is clear that the  $\alpha$ -naphtholphthalein determinations show a much smaller dispersion even though the workers had a very extensive experi-

ence with phenolphthalein and practically none with  $\alpha$ -naphtholphthalein. The standard deviations of the  $\alpha$ -naphtholphthalein titrations are significantly less than those of the phenolphthalein titrations. Furthermore, the "true" acid content values as determined by potentiometric titrations check very closely with the  $\alpha$ -naphtholphthalein titration values and depart significantly from the phenolphthalein values.

#### SUMMARY

1. The aging of whiskey results not only in an increase in the total acid content but also in an increase in the weak acids present with consequent flattening of the titration curves.

2. Above concentrations of 10 per cent by volume, the effect of ethanol upon the titration curve is appreciable. For a reproducible technic, it is essential that the sample be diluted to 10 per cent ethanol or less.

3. The effect of diminishing the acid concentration upon the titration curve is small.

4. The effect of added indicators upon the titration curves may be considerable;  $\alpha$ -naphtholphthalein offers positive advantages over phenolphthalein, having a greater color contrast and changing color below the inflection point of the titration curve.

5. The precision of the total acid titration when internal indicators are used is approximately  $\pm 0.5$  per cent, and it was demonstrated that such precision can be attained in practical work by use of optimum conditions.

6. The precision of the volatile acid titration when internal indicators are used is about  $\pm 0.3$  per cent.

7. The fixed acid determination is the least precise, and when internal indicators are used, will not be better than  $\pm 2$  per cent.

8. The fixed acid determination, when phenolphthalein is used, may be inaccurate by 25 per cent or more.

9. The component fixed and volatile acid contents are dependent upon the method of preparation of the sample. In addition, the fixed acid is subject to destruction when the residue fraction is heated.

10. The significance of the official A.O.A.C. fixed acid determination is doubtful.

#### ACKNOWLEDGMENT

The authors are indebted to the members of the Analytical Division of the Schenley Research Institute for their assistance in the potentiometric and indicator titrations.

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## DIFFERENTIAL ACTION OF PERMANGANATE AND CERIC SULFATE ON CUPROUS OXIDE PREPARED IN PRESENCE OF IODIDE\*

By J. T. SULLIVAN†

Jackson and McDonald<sup>1</sup> have recently called attention to the contamination of cuprous oxide by organic decomposition products during sugar analysis. Another case of contamination of cuprous oxide has been observed and may be pointed out.

In adapting the method of Phillips<sup>2</sup> to the determination of fructose in the presence of other sugars by measuring the reducing power of the fructose after oxidation of aldoses by iodine, the writer noted that different titration values were obtained when ceric sulfate was used instead of permanganate. No great difference was observed between the titrations by these two oxidizing agents if iodide was not present during the reduction of copper. With no other difference occurring in the procedures to affect the titrations, it appeared that there must be a difference in the action of the two oxidants toward the copper precipitate prepared when iodide was present. The following tests were carried out to demonstrate this fact.

Four lots of cuprous oxide were prepared by heating reducing sugar with the copper reagent; removing the copper precipitate; washing it with water, alcohol, and ether; and drying it in a vacuum desiccator. In the preparation of two of the cuprous oxide samples, Nos. 3 and 4, the sugar solution contained 0.672 per cent potassium iodide; in the preparation of the other samples iodide was not present. Lots 1 and 3 were prepared by heating fructose, and the cuprous oxide was removed by filtration on sintered glass crucibles. Lots 2 and 4 were prepared with invert sugar, and the cuprous oxide was separated by centrifuging.

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<sup>1</sup> *This Journal*, **25**, 988 (1942).

<sup>2</sup> *Ibid.*, **24**, 181-182 (1941).

TABLE 1.—*Analysis of Cu<sub>2</sub>O prepared in absence and in presence of KI*

SAMPLE NUMBER	METHOD OF PREPARATION	Cu <sub>2</sub> O TITRATED, AS Cu, AS DETERMINED ELECTROLYTICALLY	Cu RECOVERED— CERIC SULFATE TITRATION		Cu RECOVERED— KMnO <sub>4</sub> TITRATION	
			mg.	per cent	mg.	per cent
1	No iodide	2.70	2.73	101.1	2.71	100.4
1	No iodide	5.40	5.43	100.6	5.39	99.8
2	No iodide	13.72	13.53	98.6	13.85	100.9
2	No iodide	17.60	17.26	98.1	17.59	99.9
Av.				99.6		100.3
3	Iodide present	2.42	2.39	98.8	2.53	104.5
3	Iodide present	4.84	4.78	98.8	5.07	104.8
4	Iodide present	10.62	10.53	99.2	11.30	106.4
4	Iodide present	19.49	19.37	99.4	20.64	105.9
Av.				99.1		105.4

Portions of each lot were weighed out, dissolved in ferric alum and a mixture of sulfuric and phosphoric acids, and different aliquots were titrated with 0.01 *N* ceric sulfate (standardized with arsenious oxide) and with 0.01 *N* potassium permanganate (standardized with sodium oxalate). The recoveries of copper from the titrations are given in Table 1. The percentage recoveries are based upon the copper content of the cuprous oxide samples as determined electrolytically.

In the titration of ordinary cuprous oxide, results close to the theoretical were obtained with both permanganate and ceric sulfate. In the titration of cuprous oxide prepared in the presence of iodide, permanganate gave about 6 per cent higher results than did ceric sulfate. In the latter case a contaminant must be present. It has not been further investigated, but it is of such a nature that it reacts with permanganate and either does not react with ceric sulfate or if it does it is to a lesser degree.

### ANALYSIS OF STOCK FEEDS FOR CALCIUM, PHOSPHORUS, AND IRON

By C. V. MARSHALL, R. B. CARSON, and T. DAVIS (Plant Products  
Division, Department of Agriculture, Ottawa, Canada)

In these laboratories the determination of calcium and phosphorus, and sometimes iron, is made on mineral supplements and on mixed feeds containing added mineral ingredients.

The chapter on Grain and Stock Feeds in *Methods of Analysis A.O.A.C.*, 1940, gives a method for calcium, but it has been observed that

low results are obtained if certain ingredients are present.<sup>1</sup> No official methods are given in this chapter for the determination of phosphorus or iron. Phosphorus has been determined according to the procedure given in the same publication for fertilizers. Since the addition of iron in some form to rations for young pigs has become quite general a standard procedure for this determination is also necessary. A colorimetric method for iron that specifies potassium thiocyanate is given under the chapter on plants.

As the official method for calcium is long and fails to recover all the calcium when present as the sulfate or monophosphate, the writers sought some method that would recover all the calcium and allow phosphorus and iron determinations to be made on the same solution, in order to save time and reagents.

The use of perchloric acid was suggested by the results obtained in wet oxidations by Gerritz,<sup>2</sup> St. John and Gerritz,<sup>3</sup> and Gieseking *et al.*<sup>4</sup> It has been shown by Susano and Barnett<sup>5</sup> that perchloric acid does not interfere with the precipitation and titration of ammonium phosphomolybdate.<sup>5</sup> The writers have used an adaptation of suggested procedures on about 1200 samples of feeding stuff over a period of several years. The method is as follows:

In a 200 ml. volumetric  $P_2O_5$  flask, digest 2 grams of sample with 10 ml. of  $HNO_3$  by boiling gently for 30 minutes, or letting stand overnight. Allow to cool somewhat and add 5 ml. of  $HClO_4$  (70–72%). Continue the digestion by boiling gently until a slight charring occurs when the acid becomes concentrated. Then raise the heat and complete the digestion until the solution is water white and dense white fumes appear. After cooling slightly, make to volume, allow to settle, and take suitable aliquots for calcium, phosphorus, and iron determinations according to the quantity of these elements present.

For calcium the A.O.A.C. method is followed, beginning "Pipet 25 ml. of clear liquid . . ." Phosphorus is determined by the volumetric method for fertilizers. For the iron determination, proceed as follows:

Place an aliquot containing ca. 0.25 mg. of iron in a 50 ml. volumetric flask. Add 5 drops of  $HNO_3$ , 5 ml. of  $HCl$ , and water to make ca. 30 ml. Bring to boil on a low hot plate and cool to room temperature. Add 5 ml. of 32%  $NH_4SCN$  to develop the color and compare in a colorimeter with a standard containing about the same quantity of iron similarly treated. ( $KSCN$  cannot be used to develop the color because of the cloudiness caused by  $KClO_4$  when a large aliquot is used.)

Five analysts used the perchloric acid digestion on a large number of samples, and no trouble was encountered when the directions as outlined were followed. *The initial digest with nitric acid should not be allowed*

<sup>1</sup> Personal communication from B. P. Sutherland, Trail, B. C.

<sup>2</sup> *Ind. Eng. Chem., Anal. Ed.*, 7, 116 (1935).

<sup>3</sup> *Ibid.*, 167.

<sup>4</sup> *Ibid.*, 185.

<sup>5</sup> *Ibid.*, 8, 183 (1936).

to go to dryness since an explosion may occur if the perchloric acid is added in the absence of nitric acid. The quantities of nitric and perchloric acid have been regulated for the amount of sample taken. No explosion need occur if the directions are followed, especially to have nitric acid present when perchloric acid is added. Of course nitric acid may be added before the perchloric acid or mixed with it, but it is preferable to start a new digest since the digestion proceeds with difficulty after it has been allowed to go to dryness.

TABLE 1.—Results on calcium and phosphorus (per cent)

SAMPLE	THEORETICAL	OFFICIAL METHOD	RECOVERY	PERCHLORIC DIGESTION	RECOVERY
Calcium					
1	—	0.75	—	0.76	—
2	7.68	4.90*	63.8	7.68	100.0
3	7.00	6.90	98.6	7.00	100.0
4	—	31.58	—	31.63	—
5	13.62	13.17†	96.7	13.45	98.8
Phosphorus					
1	—	0.63	—	0.63	—
2	12.52	12.59	100.5	12.59	100.5
3	0.49	0.49	100.0	0.49	100.0
4	—	14.36	—	14.41	—
5	8.08	8.16	101.0	8.14	100.7

\* Theoretical recovery could not be obtained even by leaching the ash and igniting again.

† By leaching 13.48% Ca was obtained, giving a recovery of 99%.

To check the method, five samples were made up to contain varying quantities of calcium and phosphorus. These samples were analyzed for calcium by the A.O.A.C. method, and for phosphorus by the A.O.A.C. volumetric method for fertilizers, which specifies the magnesium nitrate ignition. Calcium and phosphorus were also determined on aliquots of a solution obtained by the perchloric acid digestion, according to the method outlined above. These results are shown in Table 1. The samples were made up as follows:

- 1.—Mixed grain.
- 2.—Mixed grain 50, calcium monophosphate 50, containing by analysis 14.6% Ca and 24.4% P.
- 3.—Mixed grain 77.8%, calcium sulfate 22.2%, containing by analysis 28.9% Ca.
- 4.—Bone meal.
- 5.—Mixed grain 50, calcium monophosphate 20, bone meal 20, and calcium carbonate 10.

It may be seen from Table 1 that the perchloric acid digestion method gave very good recoveries of calcium, whereas the A.O.A.C. method was

TABLE 2.—*Results on calcium by two methods*

SAMPLE	PROPOSED METHOD	OFFICIAL METHOD
	per cent	per cent
Commercial mineral conc.	15.8	13.6
Commercial mineral conc.	20.0	20.2
A } Used in A.O.A.C. collaborative work in	36.0	36.0
B } 1938-39, <i>This Journal</i> , 22, 647 (1939)	20.9	21.0
C }	14.9	14.9

slightly low with samples containing calcium sulfate and quite low where much monophosphate was present. There is no significant difference between phosphorus recoveries by the official magnesium nitrate ignition and the perchloric acid digestion.

TABLE 3.—*Results on iron*

SAMPLE	IRON IN 2 GRAMS	IRON ADDED	IRON FOUND	IRON RECOVERED	
	mg.	mg.	mg.	mg.	per cent
1	0.50	5.00	5.78	5.28	105.6
2	0.38	5.00	5.38	5.00	100.0
3	0.42	5.00	5.50	5.08	101.6
4	2.06	5.00	7.22	5.16	103.2
5	0.72	5.00	5.78	5.08	101.6

Table 2 shows the results for calcium obtained on five samples by the official and perchloric acid digestion methods. The first commercial mineral concentrate was known to contain calcium monophosphate.

Iron was also determined on the samples reported in Table 1, by the method outlined. To check recoveries, a standard solution containing 5 mg. of iron was added to duplicate samples before digestion. These results are outlined in Table 3.

TABLE 4.—*Results on iron in special mixed feed*

IRON ADDED	IRON FOUND	IRON RECOVERED		IRON IN SAMPLE
mg.	mg.	mg.	per cent	per cent
—	1.71	—	—	0.086
2.50	4.13	2.42	96.8	0.207
4.00	5.65	3.94	98.5	0.283
10.00	12.00	10.29	102.9	0.600
15.00	16.90	15.19	101.3	0.85
25.00	27.13	25.42	101.7	1.36

A further check on the recovery of iron was made on a mixed feed containing meat meal, fish meal, soybean oil meal, wheat germ, buttermilk powder, alfalfa meal, steamed bone meal, salt, potassium iodide, cod liver oil, limestone, and manganese sulfate. A 2 gram sample was placed in a digestion flask, and a standard solution containing varying quantities of iron was added. The digestion and analysis then proceeded as usual. These results are shown in Table 4.

#### SUMMARY

- (1) A method that allows all three determinations to be made on aliquots of one digestion is suggested for the determination of calcium, phosphorus, and iron in mineral supplements and in feeds.
- (2) Complete recovery of the calcium in calcium sulfate and calcium monophosphate was obtained.
- (3) Iron recoveries varied from 96.8 to 105.6 per cent.
- (4) Compared with the official method, the proposed method for preparation of solutions results in a substantial saving of time and material.

#### DETERMINATION OF CHLORINE AND BROMINE

By G. R. CLARK and J. H. JONES (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

Lang<sup>1,2</sup> described a method for the determination of bromides in the presence of chlorides by oxidation with permanganate in a phosphoric acid solution containing cyanide. Under these conditions the bromide is quantitatively converted to bromine cyanide, while the chloride is not oxidized. He reduced the excess permanganate with ferrous ammonium sulfate, added potassium iodide, and titrated the liberated iodine with thiosulfate.

Chloride and bromide ions can be determined in the presence of each other by precipitating the combined silver halides from one portion of the sample and determining the bromide in another portion by Lang's method. Bromine is thus determined directly and chlorine by difference.

The method to be described for bromine is a modification of the Lang method. It seemed desirable to use either the wet oxidation method recommended by Jones<sup>3</sup> or that of Thompson and Oakdale<sup>4</sup> for the decomposition of organic compounds. Jones recommends sulfite and Thompson and Oakdale propose arsenite as an absorbent in the bromine determination. Both were investigated, but each appeared to have adverse effects

<sup>1</sup> *Z. anorg. Chem.*, **144**, 75 (1925).

<sup>2</sup> Kohlthoff, and Furman, "Volumetric Analysis," Vol. II, p. 383, New York, John Wiley & Sons (1929).

<sup>3</sup> *This Journal*, **25**, 944 (1942).

<sup>4</sup> *J. Am. Chem. Soc.*, **52**, 1195 (1930).



on the recovery. A 1 per cent solution of hydrazine sulfate was found to be an efficient absorbent for the halogens, and it did not affect the results.

The absorbent was tested by the oxidation of mixtures of pure potassium bromide and sodium chloride, with salicylic acid as organic matter. Results are shown in Table 1.

TABLE 1.—*Use of hydrazine sulfate as absorbing solution*

METHOD	CHLORINE		BROMINE	
	CALCULATED gram	FOUND gram	CALCULATED gram	FOUND gram
I	0.1051	0.1046	0.1603	0.1592
	0.0390	0.0389	0.1106	0.1105
II	0.2063	0.2059	0.3133	0.3135
	0.1119	0.1117	0.2765	0.2765

I—Method recommended by Jones,<sup>3</sup> 25 ml. of 1% hydrazine sulfate as absorbent.

II—Method of Thompson and Oakdale,<sup>4</sup> 100 ml. of 1% hydrazine sulfate as absorbent.

The potassium bromide was prepared by two recrystallizations of reagent-grade potassium bromate from water, followed by decomposition of the dried crystals by careful heating over a flame,<sup>5</sup> after which the material was placed in a furnace at 400°C. for 8 hours, and finally thoroughly ground in a mortar. A portion was tested for purity by precipitation of the bromide with silver nitrate. Found: 100.0 per cent and 100.1 per cent.

Reagent-grade sodium chloride was dissolved in water and reprecipitated with hydrogen chloride gas. It was dried at 180°C. and tested by precipitation with silver nitrate. Found: 100.0 per cent, 100.1 per cent.

Prior to use all organic compounds were recrystallized to constant melting point.

#### METHOD

##### A. Bromine Determination

#### REAGENTS

*Potassium permanganate solution.*—Dissolve 3 grams of  $\text{KMnO}_4$  in 100 ml. of water.

*Potassium cyanide solution.*—Dissolve 3 grams of KCN in 100 ml. of water.

*Starch indicator solution.*—A 0.5% solution of soluble starch in water.

*Standard thiosulfate solution.*—A 0.05 *N* solution of  $\text{Na}_2\text{S}_2\text{O}_3$  in water.

*Phosphoric acid.*—85%.

*Ferrous ammonium sulfate.*—Solid  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ .

#### DETERMINATION

Dissolve the sample in 100 ml. of water in a 500 ml. iodine flask, and add ca. 10 ml. of  $\text{H}_3\text{PO}_4$ , 5 ml. of the KCN, and 10–15 ml. of the  $\text{KMnO}_4$ . Stopper the flask,

<sup>5</sup> Kohlthoff and Furman, *loc. cit.*, p. 200.

mix by gentle swirling, and allow to stand for ca. 7 minutes. Add 2-3 grams of the  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ , wash down the stopper and sides, and mix.

Add additional  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  if necessary, until the  $\text{KMnO}_4$  is completely reduced. (An excess of as much as 2 grams of the ferrous salt does not affect the results.) Wash down stopper and sides of the flask with water, add 2 grams of solid KI, and titrate rapidly with the  $\text{Na}_2\text{S}_2\text{O}_3$  with starch as an indicator.

1 ml. of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3 = 0.001998$  gram of Br.

A blank determination should be made on the reagents used. The writers found that the blank varied with the quantity of cyanide added. With 5 ml. of the 3 per cent solution, the blank was equivalent to 0.1 ml. of 0.05 N sodium thiosulfate. Recrystallization of the potassium cyanide from water eliminated the blank. Results of analyses of pure potassium bromide by this method appear in Table 2. Table 3 shows the results of analyses of potassium bromide in the presence of sodium chloride.

TABLE 2.—*Bromine in pure potassium bromide*

KBr USED	BROMINE		RECOVERY
	CALCULATED	FOUND	
gram	gram	gram	per cent
0.0886†	0.0595	0.0594	99.8
0.0886*†	0.0595	0.0595	100.0
0.0886†	0.0595	0.0595	100.0
0.0709*†	0.0476	0.0475	99.8
0.0709†	0.0476	0.0476	100.0
0.1254	0.0842	0.0842	100.0
0.1481*	0.0994	0.0995	100.1
0.1101	0.0739	0.0737	99.8
			Av. 99.9

\* Aliquot portions of a standard solution.

† 30 ml. of a 1% hydrazine sulfate solution added.

TABLE 3.—*Bromine in pure potassium bromide in presence of NaCl*

KBr PRESENT	NaCl PRESENT	BROMINE		RECOVERY
		CALCULATED	FOUND	
gram*	gram	gram	gram	per cent
0.1000	0.1000	0.06715	0.06696	99.7
0.1000	0.1000	0.06715	0.06708	99.9
0.1000	0.5000	0.06715	0.06716	100.0
0.1000	1.000	0.06715	0.06715	100.0
0.1000	2.000	0.06715	0.06704	99.8
				Av. 99.9

\* Aliquot portions of standard solution.

### B. Chlorine and Bromine in Soluble Inorganic Compounds

Dilute the dissolved sample to a definite volume with water, and in an aliquot portion determine the bromine as directed in A. From another aliquot precipitate the halides with  $\text{AgNO}_3$  in the usual manner. From the bromine found in A calculate the weight of  $\text{AgBr}$  in the precipitate and subtract from the total weight of the precipitate. From the difference (the weight of  $\text{AgCl}$ ) calculate the  $\text{Cl}$  present.

$$\text{Br} \times 2.3499 = \text{AgBr};$$

$$\text{AgCl} \times 0.24737 = \text{Cl}.$$

Results of analysis by this method appear in Table 4.

TABLE 4.—Chlorine and bromine in presence of each other

BROMINE		CHLORINE		RECOVERY	
CALC.	FOUND	CALC.	FOUND	BR.	CL.
gram	gram	gram	gram	per cent	per cent
0.1343*	0.1340	0.1266	0.1270	99.8	100.3
0.1190	0.1186	0.1213	0.1221	99.7	100.6
0.1343*	0.1347	0.0607*	0.0606†	100.2	99.9
0.1461	0.1460	0.00092*	0.00093†	99.9	101.0
0.0815	0.0817	0.00061*	0.00058†	100.2	95.0

\* Aliquot portions of standard solutions of pure  $\text{KBr}$  and  $\text{NaCl}$ .

† Precipitate weighed on a semimicro balance.

### C. Chlorine and Bromine in Organic Compounds

Samples of 2,5-dichloroaniline, 4-bromoacetanilide, 2,5-dichloro-4-bromoacetanilide, and 4-chloro-2,6-dibromo-resorcinol were analyzed. These compounds were oxidized as described by Jones.<sup>3</sup> A 1 per cent hydrazine sulfate solution was used to absorb the liberated halogens. After

TABLE 5.—Bromine and chlorine in organic compounds

	SAMPLE WT.	BROMINE		CHLORINE	
		FOUND	CALC.	FOUND	CALC.
	gram	per cent	per cent	per cent	per cent
4-bromoacetanilide	0.2525	37.30	37.36	—	—
2,5-dichloroaniline	0.1110	—	—	43.83	43.78
2,5-dichloroaniline and 4-bromoacetanilide	0.0310	37.38*	37.36	43.79†	43.78
2,5-dichloroaniline and 4-bromoacetanilide	0.1037	37.38*	37.36	43.67†	43.78
2,5-dichloroaniline and 4-bromoacetanilide	0.2804	—	—	—	—
2,5-dichloroaniline and 4-bromoacetanilide	0.0414	37.27*	37.36	43.86†	43.78
2,5-dichloro-4-bromoacetanilide	0.2863	28.22	28.25	24.95	25.07
2,5-dichloro-4-bromoacetanilide	0.2461	28.19	28.25	25.05	25.07
4-chloro-2,6-dibromoresorcinol	0.1912	52.60	52.86	11.80	11.73
4-chloro-2,6-dibromoresorcinol	0.1717	52.86	52.86	11.74	11.73

\* Calculated on the quantity of 4-bromoacetanilide taken.

† Calculated on the quantity of 2,5-dichloroaniline taken.

the oxidation was completed, the absorbent solution was diluted to 110 ml., and the determination was carried out as directed under B. Results are given in Table 5.

#### SUMMARY

Methods for the determination of chlorine and bromine in the presence of each other are described. Preliminary decomposition of organic compounds by the method recommended by Jones or by the Thompson-Oakdale method is satisfactory. A hydrazine sulfate solution is used to absorb the liberated halogens. Typical results are given.

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### CHLORINE IN ASH OF FRUIT PRODUCTS

By R. A. OSBORN (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The study made by Doris Tilden (*This Journal*, 11, 216) established the groundwork for the determination of chlorine in fruits and fruit products and in plants (*Methods of Analysis*, A.O.A.C., 1940). Before ashing she added sodium carbonate as a chlorine fixative in a ratio of at least five of carbonate to one of chloride, and determined chlorine after ashing by conventional gravimetric or volumetric procedures.

In *Methods of Analysis*, A.O.A.C., 1940, paragraph 13 in Chapter XXVI refers back to "XII, 35 and 37," two satisfactory procedures for chloride determination, but this direction may cause the beginner to assume inadvertently that the ash under XXVI, 9, can be used. However, the experiment described herein indicates the need for sodium carbonate as a fixative for fruit products, notwithstanding the fact that fruit ash normally contains approximately 75 per cent of potassium carbonate, which should, and no doubt does, act to reduce loss of chlorine during ashing.

A commercial sample of canned orange juice was used to study ashing procedures and chlorine recoveries. Table 1 gives data for ash and chloride both with and without sodium carbonate as a fixative, without addition of sodium chloride to the sample. It is not surprising to find the same percentage of sodium chloride with and without the addition of sodium carbonate as a fixative in view of the small quantity of natural chloride present. As regards the ash determination the omission of the fixative is simpler manipulatively, and it is believed the ash value is more nearly accurate since no correction is required for a weight of blank, which in this instance is more than eight times the weight of the ash of the sample.

However, Pruitt (*This Journal*, 24, 397; 25, 447) mentions the presence of added sodium chloride in fruit products such as jams, jellies, and other fruit and sugar mixtures through the addition of invert sugar (inverted with hydrochloric acid, which is later neutralized with sodium bicar-

bonate). Further, in canning operations apples, peaches, quinces, and other fleshy fruits are often immersed in salt solution in order to retard oxidative or enzymatic changes that result in fruit darkening. As is indicated later, significant amounts of salt may be absorbed by the fruits so immersed. In order to evaluate fruit content from its ash and ratio of ash to potash, phosphate, or other fruit constituents, it is obviously desirable to make the calculation after correction has been made for added salt. Since natural sodium chloride in fruits is seldom more than a few hundredths per cent, the calculation is not materially affected where salt is

TABLE 1.—*Ash and chloride results on orange juice obtained with and without added  $\text{Na}_2\text{CO}_3$  and without added  $\text{NaCl}$*

PROCEDURE*	WEIGHT OF SAMPLE	ASH	ASH CORRECTED FOR BLANK	CHLORIDE AS $\text{NaCl}$	
	grams	gram	per cent	gram	av. per cent
Ash, XXVI, 9, or XXXIV, 9	15.1610	0.0610	0.40	0.0012	
Cl, XII, 35	15.5094	0.0624	0.40	0.0011	0.008
Ash, XXVI, 9, or XXXIV, 9, +10 ml. 5% $\text{Na}_2\text{CO}_3$	14.9928	0.5505	0.37	0.0012	
Cl, XII, 35	15.5000	0.5532	0.37	0.0012	0.008
Ash, XXVI, 9, +10 ml. 5% $\text{Na}_2\text{CO}_3$	Blank	0.4956	—	+0.0001	
Cl, XII, 35	Blank	0.4961	—	-0.0001	

\* References are to *Methods of Analysis*, A.O.A.C., 1940.

an added ingredient, if total sodium chloride is considered as added sodium chloride. Ashing should be carried out without loss of chlorine in order to make proper correction of ash for added sodium chloride since the sodium of the sodium chloride in all probability is not volatilized but is converted to carbonate.

The necessity of a fixative such as sodium carbonate during ashing of fruit samples containing added sodium chloride is indicated in Tables 2 and 3. In Table 2, 0.0117 gram of sodium chloride (2 ml. 0.1 *N* sodium chloride) was added to portions of the commercial orange juice used for data in Table 1, and duplicate samples were ashed without an ash aid, with additions of 0.5000 gram of sodium carbonate (10 ml. 5% solution—blank of 0.4958 gram), and with 0.5000 gram of magnesium nitrate (10 ml. 5% solution—blank 0.0804 gram). Magnesium nitrate was tried since a magnesium salt is used to speed up the ashing of cereal products (*Methods of Analysis*, A.O.A.C., 1940, 212, 7), and since Gerritz (*This Journal*, 25, 232) employed it as an ash aid for the rapid determination of potash in fruits and fruit products.

The data in Table 2 indicate 98 per cent recovery of chloride with sodium carbonate, 50 per cent with magnesium nitrate, and 87 per cent when no ash aid was employed. The material loss of chloride with the magnesium salt is in harmony with the findings of Browne and Gamble,<sup>1</sup> who observed 100 per cent loss of chlorine when magnesium chloride was incinerated with sugar.

TABLE 2.—*Results with orange juice showing necessity for Na<sub>2</sub>CO<sub>3</sub> fixative*

PROCEDURE	WEIGHT OF SAMPLE	ADDED NaCl	ASH	ASH CORRECTED	CHLORIDE AS NaCl	NaCl FOUND	NaCl AT START*
				FOR ADDED NaCl AND ASH AID			
	grams	gram	gram	per cent	gram	per cent	per cent
Ash, XXVI, 9	15.2544	0.0117	0.0700	0.38	0.0111	0.073	0.084
or XXXIV, 9	15.3185	0.0117	0.0702	0.38	0.0112	0.073	0.084
Cl, XII, 35							
Ash, XII, 34							
Cl, XII, 35	15.4216	0.0117	0.5665	0.38	0.0126	0.082	0.084
+10 ml. 5% Na <sub>2</sub> CO <sub>3</sub>	15.2765	0.0117	0.5657	0.38	0.0126	0.083	0.084
Ash, XXVI, 9, +10 ml. 5% Mg(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	15.3580	0.0117	0.1530	0.40	0.0064	0.042	0.084
Cl, XII, 35, no Na <sub>2</sub> CO <sub>3</sub>	15.2220	0.0117	0.1524	0.40	0.0064	0.042	0.084
Ash, XXVI, 9, +10 ml. 5% Mg(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Blank	None	0.0803	—	+ .0001	—	—
Cl, XII, 35	Blank	None	0.0805	—	— .0001	—	—

\* Calculated—sum of % added NaCl + % naturally present (.008).

Table 3 contains ash and chlorine data obtained from analyses of a commercially prepared canned quince and a commercial quince and sugar mixture prepared from this fruit. In the commercial preparation of the fruit, two sodium chloride solutions were used,<sup>2</sup> one between the slicing and sorting and trimming (75 pounds of sodium chloride to 100 gallons of water), and the second in blanching (10 pounds of sodium chloride to 100 gallons of water), representing approximately 8.3 per cent and 1.1 per cent solutions of sodium chloride, respectively. In Table 3 there is observed the need for a chlorine fixative during ashing of the fruit sample and the fruit and sugar sample. It appears that the sodium carbonate held

<sup>1</sup> *Facts About Sugar*, 17, 552 (1923).

<sup>2</sup> Private communication, J. A. Reeves, Inspector, U. S. Food and Drug Administration, Buffalo, N. Y.

TABLE 3.—Ash and chlorine data obtained with commercially prepared fruit products

PROCEDURE	PRODUCT	WT. SAMPLE	ASH	Cl as NaCl
		grams	per cent	per cent
		No fixative		
Ash, XXVI, 9	Quince	12.0490	0.46	0.180
Cl, XII, 35	Fruit	14.9810	0.45	0.175
Ash, XXVI, 9	Quince and	16.0434	0.23	0.033
Cl, XII, 35	Sugar	16.2575	0.23	0.034
		Na <sub>2</sub> CO <sub>3</sub> fixative added		
			WT. ADDED Na <sub>2</sub> CO <sub>3</sub> grams	
Cl, XII, 35	Quince	17.3815	1.6455	0.228
	Fruit	14.0130	1.7380	0.228
Cl, XII, 35	Quince and	22.4605	1.4600	0.110
	Sugar	14.7038	1.9517	0.108
		Na <sub>2</sub> CO <sub>3</sub> fixative + 0.0200 gram of added NaCl		
Cl, XII, 35	Quince	15.0582	1.0083	0.228*
	Fruit	15.9013	1.0187	0.228*
Cl, XII, 35	Quince and	15.3940	1.0170	0.112*
	Sugar	18.4523	1.0363	0.111*

\* Calculated after subtraction of added NaCl (0.0200 gram).

the chloride since addition of 20 mg. of sodium chloride to the samples gave 20 mg. greater recovery. When no fixative was used the fruit sample gave but 77 per cent chloride recovery, while the fruit and sugar mixture gave chloride recovery of only 30.7 per cent.

It is concluded that it is expedient to use sodium carbonate as a chlorine fixative during ashing for fruits and fruit products in all instances where the quantity of chloride in the sample is material.

## VOLUMETRIC DETERMINATION OF IODINE IN IODIZED SALT

By R. A. OSBORN, A. E. MIX, and L. L. RAMSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

The use of bromine or bromine water in weak acid solution as the reagent for oxidation of the iodide in a prepared sample to the iodate was proposed by Schulek<sup>1</sup> and later adopted by Elmsie-Caldwell,<sup>2</sup> Sadusk and

<sup>1</sup> *Z. anal. Chem.*, 66, 161 (1925); *Z. anorg. allgem. Chem.*, 43, 184 (1909); *Pharm. Zentralhalle*, 64, 511 (1923).

<sup>2</sup> *This Journal*, 18, 338 (1925); 21, 86 (1938).

Ball,<sup>3</sup> and by Kainrath.<sup>4</sup> Excess bromine is removed by boiling<sup>2,3</sup>, by phenol<sup>1</sup>, or by formic acid<sup>4</sup>, after which six equivalents of free iodine are liberated for each original unit of iodide by addition of an excess of potassium iodide. The measurement is completed by titration with standard thiosulfate with starch as indicator. In the procedure developed by the writers, a definite quantity of bromine in water is used as the oxidizing reagent, and the slight excess of bromine is removed in part with dilute sulfite solution and the remainder with phenol in such a manner as to prevent formation of the objectionable precipitate of tribromophenol.\* The determination is made at room temperature. The procedure is simple and rapid and results obtained by its use are accurate and reproducible.

The method follows:

#### VOLUMETRIC METHOD—IODINE IN SALT

##### REAGENTS

*Methyl red indicator.*—Dissolve 1 gram of methyl red in 200 ml. of 50% alcohol and filter if necessary.

*Sulfuric acid solution.*—1+19 dilution of A.C.S. reagent.

*Sodium thiosulfate solution.*—0.01 N. Recently standardized.

*Phenol solution.*—5% U.S.P.

*Potassium iodide solution.*—10% A.C.S.

*Soluble starch solution.*—1%.

*Sodium sulfite solution.*—1% A.C.S.

*Bromine water.*—Prepare a strong bromine water solution A.C.S. reagent-grade bromine and distilled water. Determine its concentration as follows: From a buret add 3–5 ml. of the bromine water to a flask containing 50 ml. of distilled water, 5 ml. of 10% KI solution and 5 ml. of 1+9 H<sub>2</sub>SO<sub>4</sub>. Titrate liberated iodine with 0.1 N thiosulfate. Express concentration as mg. Br/ml.

*Control solutions of potassium iodide.*—Dissolve 0.3280 gram of dry A.C.S. reagent-grade KI in distilled water and make up to 250 ml. in a volumetric flask. 1 ml. of the solution = 1 mg. of iodine and 1.312 mg. of KI. Prepare a 1 to 5 control solution.

*Sodium chloride solution.*—20% A.C.S. reagent grade.

##### DETERMINATION

Dissolve 50 grams of the salt sample in distilled water and make up to 250 ml. in a volumetric flask. Filter if necessary and add 50 ml. aliquots to 200 ml. Erlenmeyer flasks. Add, dropwise, sufficient 1+9 H<sub>2</sub>SO<sub>4</sub> to make the samples acid to methyl red indicator. Add bromine water dropwise from a buret in an amount equivalent to 20 mg. of bromine.† After a few minutes destroy the greater portion of the remaining free bromine by adding the Na<sub>2</sub>SO<sub>3</sub> solution *dropwise while mixing*. Wash down neck and sides of flask with distilled water and complete the removal of free bromine by the addition of 1 or 2 drops of phenol solution. Add 1 ml. of 1+9 H<sub>2</sub>SO<sub>4</sub> and 5 ml. of 10% KI and titrate the liberated iodine with 0.01 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution, adding 1 ml. of the starch indicator near the end of the titration.

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 5, 386 (1933).

<sup>4</sup> *Z. anal. Chem.*, 125, 1 (1942).

\* The combination of sulfite and a few drops of phenol for reduction of bromine while leaving the element to be determined in an oxidized condition for subsequent volumetric determination was used by Osborn in a procedure for the determination of selenium in foods (*This Journal*, 20, 194 (1937)).

† This is sufficient for all commercial iodized salt samples.



Correct the determination for a blank on the reagents and make one or more control determinations, using 50 ml. of the NaCl solution to which is added appropriate quantities of the dilute control KI solution. 1 ml. of 0.01 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 0.2116 mg. of iodine and 0.2776 mg. of KI.

Table 1 shows results of duplicate analyses of nine 20 per cent weight volume salt solutions containing varying quantities of iodide. The solu-

TABLE 1.—*Collaborative results—iodine in salt solutions*  
(Per cent as KI)

SAMPLE NO.	A.E.M.	R.A.O.	L.L.R.
1	0.0141	0.0140	0.0141
	0.0141	0.0141	0.0141
2	0.0225	0.0225	0.0227
	0.0225	0.0224	0.0228
3	0.0111	0.0112	0.0113
	0.0111	0.0112	0.0111
4	0.0200	0.0200	0.0204
	0.0199	0.0200	0.0204
5	0.0208	0.0208	0.0208
	0.0208	0.0208	0.0210
6	0.0161	0.0160	0.0161
	0.0161	0.0159	0.0160
7	0.0117	0.0116	0.0116
	0.0117	0.0115	0.0117
8	0.0228	0.0228	0.0228
	0.0228	0.0227	0.0229
9	0.0272	0.0270	0.0268
	0.0272	0.0272	0.0268
	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
0.01 <i>N</i>	0.05	0.05	0.07
Blank	0.05	0.05	0.09

tions were analyzed by each of the writers according to this procedure, and each standardized his 0.01 *N* thiosulfate and corrected the results for a blank determination.

Table 2 indicates recoveries with this procedure with varying quantities of the dilute control potassium iodide solution added to 50 ml. of 20 per cent solution of reagent-grade salt. All determinations are corrected for the blank on the reagents. The samples were given to the analysts as unknowns.

TABLE 2.—*Recoveries of iodine added as KI to 50 ml. of 20% salt solutions*

ANALYST 1 MG. I <sub>2</sub>		ANALYST 2 MG. I <sub>2</sub>		ANALYST 3 MG. I <sub>2</sub>	
ADDED	FOUND	ADDED	FOUND	ADDED	FOUND
0.10	0.12	0.10	0.10		
0.10	0.12	0.10	0.10		
0.60	0.61	0.30	0.29	0.40	0.38
0.60	0.62	0.30	0.30	0.40	0.39
1.10	1.11	0.40	0.40	1.00	0.98
1.10	1.11	0.40	0.40	1.00	0.98
1.50	1.48	0.65	0.65	2.00	1.99
1.50	1.50	0.65	0.66	2.00	2.00
1.80	1.82	2.00	1.95	2.00	1.97
1.80	1.82	2.00	1.95	2.00	1.99
Blank	0.01	Blank	0.01	Blank	0.01
Blank	0.01	Blank	0.01	Blank	0.01

The use of too small or too great an excess of bromine in water must be avoided since either condition may cause low results. This is illustrated in Table 3. It was found that under the conditions of the experiment 10 mg. of bromine is in all probability insufficient, although by calculation 8 mg. of bromine should be sufficient for the oxidation. Recoveries of iodide were definitely low when more than 60 mg. of bromine was added. Iodate may be reduced by the greater concentration of hydrobromic acid, forming free bromine, which is bound by the phenol.

Unless the sulfite solution is added slowly and with thorough mixing, low recoveries of iodide may occur due, doubtless, to reduction by the sulfite of a portion of the iodate. For example, when the regular procedure

TABLE 3.—*Effect of quantity of bromine added on recoveries of iodine with 10 grams of salt in 50 ml. water + 2.00 mg. of iodine as KI*

ADDED BROMINE IN WATER mg.	RECOVERY mg. I <sub>2</sub>	Mg. ADDED BROMINE IN WATER mg.	RECOVERY mg. I <sub>2</sub>
10	1.81	70	1.95
10	1.92	70	1.87
20	2.00	80	1.97
20	2.00	80	1.96
40	2.01	100	1.80
40	2.00	100	1.83
50	2.02	130	1.70
50	2.01	130	1.86
60	1.99	20 to Blank	0.01
60	2.00	20 to Blank	0.01

was used, and 2 mg. of iodine as potassium iodide was added to 50 ml. of the 20 per cent salt solution recoveries of 2.00 and 2.01 mg. were obtained, but when the experiment was repeated and the same quantity of sulfite was added before stirring, recoveries of 1.80 and 1.86 mg. of iodine resulted.

## THE POLYBASIC ACIDS OF FRUITS AND FRUIT PRODUCTS

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The official methods (*Methods of Analysis*, A.O.A.C., 1940, pp. 341-347) for the determination of the organic polybasic acids of fruits and fruit products are involved and time-consuming. This is particularly true of the methods for laevo and inactive malic acids. Furthermore, the chapter does not provide methods for a number of acids, the diagnostic values of which are indispensable for judging the nature of a fruit or fruit product.

The object of this paper is to furnish serviceable methods for the determination of the important organic polybasic acids, natural or added, that may occur in the various types of products appearing in the market. Particular attention was devoted to the time element, and it is now possible without sacrifice of accuracy to determine many of the acids in considerably less time than was heretofore required.

The acid components of fruits and fruit products may be divided into two general groups, monobasic and polybasic. The members of the two groups are predominantly organic; the inorganic members, sulfuric, phosphoric, and hydrochloric acids, are present in natural fruits in small quantities only. No consideration is given here to the latter acids since accurate methods for their determination are now available.

The organic polybasic acids occurring naturally in the common fruits are laevo malic, normal citric, isocitric, tartaric, tannic, succinic, and oxalic. In addition to these, inactive malic acid may be present as an added ingredient. Oxalic and succinic acids occur only sparingly in the common fruits but in fermented beverages the quantity of succinic acid may reach considerable proportions.

All of these acids are readily soluble in alcohol and are precipitable quantitatively as the lead salts from strong alcohol. Pectin also belongs to this group, but it differs from the acids mentioned in that it is precipitated from weak acid solution with strong alcohol.

While the main portion of the acids exists in fruits in the free state, a considerable part is present in the form of salts and esters. The methods

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described in this paper determine the *total* acid content, free and combined.

Pectin, tannin, and coloring matters are present in all fruits; laevo malic and normal citric acids occur almost universally;<sup>1</sup> tartaric acid has been identified with certainty only in the grape and the tamarind; isocitric acid has been reported in the common fruits only in the blackberry and its hybrids;<sup>2</sup> and inactive malic acid (racemic malic) has not been reported in fruits, but its presence in a fruit product is due solely to the addition of the commercial preparation.

Fruit products may contain the following monobasic acids: Acetic and possibly other members of the fatty acid series, lactic, benzoic, salicylic, quinic, glycolic, and glyoxylic. In the free state all of these acids form alcohol-soluble lead salts, a circumstance that offers a simple means for their separation from the members of the polybasic group. It is not the intention to consider at this time methods for the determination of monobasic acids since satisfactory procedures for the more important members are now available.

Before a description of methods for the determination of polybasic acids is presented, a review of their properties and analytical behavior, together with a brief discussion of the steps involved in their isolation and ultimate determination, seems desirable. Based upon their behavior toward polarized light the organic components of fruits and fruit products fall into two well-defined groups, optically active and optically inactive substances. Sugars, pectin, tartaric, laevo malic, tannic, and isocitric acids turn the plane of polarized light; racemic malic, normal citric, succinic, and oxalic acids are optically inactive.

Tartaric acid is the only fruit acid that forms an alcohol-insoluble acid potassium salt; normal citric acid is the only fruit acid that yields pentabromacetone; succinic acid is the only polybasic fruit acid that resists oxidation with potassium permanganate; tannic acid in neutral solution is quantitatively separated from all other fruit acids by adsorption on carbon; oxalic acid forms lead and calcium salts that are practically insoluble in weak acetic acid solution.

Laevo and inactive malic acids when oxidized in the presence of potassium bromide form volatile bromine compounds that yield insoluble condensation products with dinitrophenylhydrazine, according to Pucher, Vickery, and Wakeman.<sup>3</sup> Normal citric, isocitric, and tannic acids also form the compound. Isocitric acid does not yield pentabromacetone, in which respect it differs fundamentally from its isomer, normal citric acid. Isocitric acid, when treated with uranyl acetate, yields a complex that displays strong dextro rotation and hence interferes with the polarimetric determination of laevo malic acid. The interference is eliminated by

<sup>1</sup> *This Journal*, 17, 527 (1934).

<sup>2</sup> *J. Am. Chem. Soc.*, 47, 668 (1925).

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 6, 288 (1934).

treating the solution with lead acetate in 2.5 per cent acetic acid solution.

Based upon the properties cited, procedures for the determination of the polybasic acids of fruits and fruit products have been promulgated.

Before subjecting a product to acid analysis it is necessary to remove from the sample solution extraneous materials such as pectin and sugars. After the removal of pectin with alcohol, the alcoholic filtrate is saponified and treated with lead acetate. The lead salts are washed free of sugars and monobasic acids with 80 per cent alcohol. The salts are suspended in water, decomposed with hydrogen sulfide gas, and filtered. The filtrate contains the total polybasic acids of the sample, organic and inorganic, in the free state in practically pure condition. With the exception of tannic acid the filtrate so obtained serves as the mother solution for all acid determinations and is hereinafter referred to as the "isolated acid solution." The solution may be used for the determination of the total acidity of the polybasic acids, free and combined.<sup>4</sup>

#### TARTARIC ACID

In principle, the procedure here proposed is essentially that described in *Methods of Analysis*, A.O.A.C., 1940. However, because of the reluctance with which the cream of tartar reaction goes to completion in the lower concentrations, a fixed weight of tartaric acid in the form of Rochelle salts is added. The substitution of nitric acid for sulfuric acid in the isolation procedure eliminates the filtration difficulties that are caused by the deposition of potassium sulfate in the strong alcohol.

#### CITRIC ACID

The official pentabromacetone procedure has been thoroughly revised, particularly that part of it that treats of the oxidation of the brominated solution. As has been shown,<sup>5</sup> the gradual addition of potassium permanganate permits a better yield of pentabromacetone, and accordingly this form of oxidation has been written into the procedure. In its present form the procedure is unusually accurate; it will definitely detect 1 mg. of the acid in 100 ml. of reaction mixture. In the calculation a correction for solubility is not necessary; the weight of pentabromacetone multiplied by the theoretical conversion factor .424 expresses grams *anhydrous* citric acid.

#### MALIC ACID (LAEVO)

Since the method here proposed is predicated on the optical rotation of the acid, it is obvious that the presence in the solution of other optically active acids interferes with the procedure. Tartaric and tannic acids are readily removed; the disturbing influence of isocitric acid, however, is not so easily eliminated or controlled. It was found that the influence of

<sup>4</sup> *This Journal*, 22, 858 (1939).

<sup>5</sup> *Ibid.*, 360.

isocitric acid is removed by a lead treatment in the presence of sodium acetate and acetic acid. This treatment is necessary only in case isocitric acid is present; in the absence of the acid the polarization is made on the isolated acid solution after the removal of tartaric acid. Unfortunately, there is no specific test for isocitric acid, and hence the only dependable

TABLE 1.—*The apparent malic acid factor .0185\**

MALIC ACID	CITRIC ACID	INACTIVE MALIC ACID	ISOCITRIC ACID	TANNIC ACID	POLARI- ZATION	MALIC ACID RETURNED	$\frac{\text{ACID}}{V^{\circ}}$
<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	$V^{\circ}$	<i>gram</i>	
.010	.050	—	—	—	— .50 — .60	.010	.0182
.025	.050	—	—	—	— 1.40 — 1.35	.025	.0182
.049	.050	—	—	—	— 2.70 — 2.65	.049	.0183
.098	.050	—	—	—	— 5.40 — 5.40	.100	.0181
.098	—	—	—	—	— 5.30 — 5.40	.099	.0183
.098	.025	—	—	—	— 5.40 — 5.40	.100	.0181
.098	.100	—	—	—	— 5.20 — 5.25	.097	.0187
.049	—	.050	.060	.075	— 2.55	.047	.0192
.074	.050	—	.045	.050	— 3.85	.071	.0192
—	.025	.050	.100	.050	+ .10	—	—

\*  $1^{\circ} V = .0185$  gram of laevo malic acid. The factor is empiric; it reflects the polarization after the 16% malic acid loss through occlusion in the Pb precipitate—.0156 + (.0156  $\times$  .16) = .0181, or .0185 approximately.

course open to the analyst is to treat the isolated acid solution as though the acid were present.

Briefly, the method for laevo malic acid involves the following steps: Removal of tartaric acid from the isolated acid solution; removal of the isocitric acid influence with lead acetate; treatment of the filtrate from the lead salts with uranyl acetate and carbon; polarization of the uranium complex in degrees Ventzke; and calculation of the grams malic acid by multiplying the  $V^{\circ}$  by the factor .0185. In the procedure a considerable

part of the malic acid is lost through occlusion in the lead precipitate and hence the factor .0185 is *apparent* only.

The data shown in Table 1 were obtained as follows:

To the aqueous solution of the acids, .05 gram of citric acid was added, and the mixture was neutralized (phph<sub>t</sub>.) with normal NaOH (let "t" = titer in ml.). To the neutralized solution 1 ml. of glacial acetic acid, 1 gram of Na-acetate, and ".25 t" gram Pb-acetate were added, and the mixture was adjusted with water to a weight of 40 grams. The flask was vigorously shaken 5 minutes and allowed to stand 10 minutes. To the mixture .5 gram of activated carbon was added, shaken 3 minutes, and allowed to stand 10 minutes. After filtration the solution was shaken 5 minutes with 2 grams of uranyl acetate and filtered. The *bright* filtrate was polarized in a 200 mm. tube in V° at 20°, white light being used.

Carbon was added to adjust the reaction to the conditions obtaining in the analysis of fruit samples and to remove the small quantity of tannic acid that remains after the lead treatment. Not only is tannic acid optically active, but its presence darkens the solution so that polarimetric readings become obscured. Any one of the many sensitized carbon preparations offered in the market is suitable. In this work "Nuchar-W" was used.

In order to determine the loss of malic acid through occlusion in the lead precipitate, the data in Table 2 are presented. They were obtained by omitting the lead treatment and hence express the polarization value of malic acid in pure solution.

TABLE 2.—*Malic acid factor in pure solution .0156\**

MALIC ACID	CITRIC ACID	POLARIZATION	MALIC ACID RETURNED	$\frac{\text{ACID}}{V^\circ}$
<i>gram</i> .0125	<i>gram</i> .050	<i>V°</i> — .80 — .75	<i>gram</i> .012	.0160
.025	—	—1.60 —1.70	.026	.0152
.049	—	—3.20 —3.20	.050	.0153
.098	.075	—6.20 —6.20 —6.30	.097	.0157

\* 1°V = .0156 gram of laevo malic acid.

The values (Table 2) were obtained in the following manner:

The acids were dissolved in water, .05 gram of citric acid was added, and the mixture was neutralized with normal NaOH. The neutralized solution was acidified

with 5 drops of glacial acetic acid and adjusted with water to a weight of 40 grams; 2 grams of uranyl acetate was added, and the mixture was shaken 5 minutes. After the addition of .5 gram of carbon, the mixture was again shaken, filtered into a 200 mm. polariscope tube, and polarized at 20°, a white light being used.

A comparison of the data in Tables 1 and 2 shows that approximately 16 per cent malic acid is lost through occlusion in the lead precipitate and that the loss is constant regardless of concentration. The data also show that the lead treatment removes isocitric acid almost quantitatively. This is clearly evidenced in the data recorded at the bottom of Table 1. In the last determination, in which malic acid is absent, the polarization indicates the presence of a small quantity of isocitric acid. Apparently the acid escaped precipitation and accounts for the slightly low polarizations and the somewhat higher malic acid factor recorded in the solutions containing the acid.

#### INACTIVE MALIC ACID (RACEMIC MALIC)

The method here proposed is an adaptation of the "hydrazine procedure" devised by Pucher, Vickery, and Wakeman<sup>3</sup> for the determination of malic acid in tobacco. These authors show that malic acids, both laevo and inactive, when oxidized in the presence of potassium bromide produce volatile bromine compounds that form insoluble condensation products with dinitrophenylhydrazine. They distil the volatile bromine compound into the hydrazine solution and evaluate the malic acid content photometrically. In the analysis of fruit products it was found that the distillation step can be omitted and the malic acid calculated from the *weight* of the hydrazine precipitate.

The hydrazine reaction is not specific for inactive malic acid. Its isomer, laevo malic acid, also produces the complex as do also normal citric, isocitric, and tannic acids. Isocitric and tannic acids are eliminated by the lead treatment described under "malic acid." The small quantity of citric acid remaining in solution is removed by the modified pentabromacetone precipitation proposed by Pucher, Vickery, and Wakeman.<sup>3</sup> The modification consists of the use of sulfur dioxide instead of iron for the removal of manganese dioxide.

In order to evaluate the inactive malic acid content, it is required to know not only the weight of the hydrazine precipitate (P) but also the weight of laevo malic acid as well as the hydrazine value of malic acid.

The values given in Table 3 were obtained in the following manner:

The acids were dissolved in 40 ml. of water, .05 gram of citric acid, .5 gram of KBr, and 3 ml. of conc.  $H_2SO_4$  were added; and the solution was heated to ca. 50°. After the mixture had stood 5 minutes, 10 ml. of 5%  $KMnO_4$  solution was added, and it was again allowed to stand 5 minutes. The mixture was then cooled to 15° and a 3%  $Na_2SO_3$  solution was added until the  $MnO_2$  was just dissolved;  $KMnO_4$  solution was then added until a slight, but distinct, brownish precipitate of  $MnO_2$  was produced. The mixture was again cooled to 15°, and after 20 grams of  $Na_2SO_3$



had been added, it was shaken 5 minutes and filtered on a Gooch crucible. To the filtrate in a beaker a few drops of the  $\text{Na}_2\text{SO}_3$  solution was added to remove the  $\text{MnO}_2$ . After the solution had been acidified with 15 ml. of conc.  $\text{HCl}$ , a solution of dinitrophenylhydrazine was added (.5 g. salt + 20 ml. of water + 10 ml. of conc.  $\text{HCl}$ , heated to boiling, and filtered), and the mixture was heated to boiling. It was then placed on the steam bath for 30 minutes, with stirring at frequent intervals, and

TABLE 3.—*Hydrazine value of malic acid .712\**

FORM OF MALIC ACID	AMOUNT TAKEN	HYDRAZINE PRECIPITATE (P)	ACID P
Inactive	gram .005	gram .0083	.602†
Inactive	.010	.0141	.709
Inactive	.020	.0280	.714
Inactive	.049	.0695	.705
Laevo	.005	.0080	.625†
Laevo	.010	.0142	.704
Laevo	.020	.0276	.725
Laevo	.049	.0695	.705
Inactive	.010	} .0276	.725
Laevo	.010		

\* 1 gram of hydrazine precipitate = .712 gram of malic acid.

† Not included in the average, since the results on concentrations lower than 10 mg. are irregular.

while still hot was filtered on a weighed Gooch crucible. The crucible was washed with hot water acidulated with  $\text{HCl}$  (100 + 5), dried 30 minutes in the water oven, and weighed.

NOTE: The .05 gram citric acid was added for the purpose of adjusting the hydrazine reaction to the conditions obtaining in the analysis of fruit products. Approximately 2 mg. of citric acid, equivalent to 1.5 mg. of hydrazine precipitate (.002 gram of malic acid), escape the pentabromacetone precipitation. The quantity is a constant and is reflected in the hydrazine value .712.

Calculation.— $.0185/.0156(.712P \times 40/10.3 - .0156V^\circ)$  or  $3.28P - .0185V^\circ$  = grams inactive malic acid in the aliquot; in which .0185 is the apparent and .0156 the actual polarization value of malic acid; .712 is the hydrazine value of malic acid; P is the grams of hydrazine precipitate and 10.3 is the average weight of the 10 ml. solution taken for the hydrazine precipitation.

### ISOCITRIC ACID

The literature does not report methods for the determination of isocitric acid. Because of the pronounced tendency of the acid to form the lactone in the course of its isolation and determination, careful attention is required to avoid all conditions that are conducive to its formation. It was found that the lactone formation proceeds rapidly when a solution of the acid is exposed to boiling temperature; at room temperature in the presence of 2 per cent acetic acid as much as 15 per cent of the acid is converted in 10 minutes.

With these considerations in mind the writer devised a method for the determination of isocitric acid that is based upon the optical rotation of the isolated acid solution (a); the optical rotation of the solution after the elimination of the isocitric acid (b); and the rotation value of isocitric acid (c).

The values given in Table 4 were obtained in the same manner as were those for malic acid shown in Table 2.

TABLE 4.—*Rotation value of isocitric acid .0295\**

ISOCITRIC ACID	CITRIC ACID	INACTIVE MALIC ACID	TANNIC ACID	POLARIZATION	ACID v°
<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	v°	
.015	.100	.050	.050	+ .50	.0300
.030	.075	.100	.050	+1.05	.0286
.060	.075	.050	.100	+2.10	.0286
.105	.075	—	.050	+3.40	.0309

\* 1°V = .0295 gram of isocitric acid.

The acid is computed from the 3 values—a, b, and c—by the following equation:

$c(a - 1.186b)$  = grams isocitric acid in the aliquot, in which c = rotation value of isocitric acid (.0295); a = polarization of the isolated acid solution in Ventzke degrees; b = polarization of laevo malic acid in Ventzke degrees; and 1.186 = correction for the malic acid occluded in the lead precipitate (.0185/.0156).

The isocitric acid used in this investigation was furnished by E. K. Nelson, deceased, formerly with the then Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C. Nelson isolated the acid as the triethyl-ester from the blackberry fruit and established the purity of the ester by the melting point of its hydrazide. For particulars concerning the isolation of the ester the reader is referred to Nelson's original contribution, "The Non-Volatile Acids of the Blackberry."<sup>6</sup> Incidentally, Nelson was the first investigator to report the occurrence of isocitric acid in nature.

The acid was prepared from the ester by saponification, precipitation of the acid as the lead salt, and liberation of the acid from its lead combination with hydrogen sulfide gas. In order to assure against lactone formation the solution was kept on the alkaline side. The limited amount of acid available necessitated fewer tests of the method. It is realized that further tests should be conducted when more of this acid is obtainable.

<sup>6</sup> *J. Am. Chem. Soc.*, 47, 568 (1925).

## TANNIN AND COLORING MATTER

The chapter on Fruits and Fruit Products (*Methods of Analysis*, A.O.A.C., 1940) does not list a method for the determination of tannin and coloring matter. The wine method, which has been used for the determination, is not suitable for samples of fruit high in solids and tannin content. An apple jelly gives no untoward difficulties; however, in the analysis of blackberry jam and similar highly colored products the titration end points are frequently so uncertain that the determinations are worthless, or mere approximations at best.

Obviously in the tannin analysis the type of product is of primary con-

TABLE 5.—*Tannin in pure solution*  
.005(b - c) = g. tannic acid

TANNIC ACID	TARTARIC ACID	CITRIC ACID	MALIC ACID	b KMnO <sub>4</sub>	c KMnO <sub>4</sub>	TANNIC ACID RETURNED
gram	gram	gram	gram	ml.	ml.	gram
.0092	—	—	—	16.1	14.7	.0070
.0092	.025	.025	.025	16.5	15.1	.0070
.0230	.050	.050	.025	20.2	15.4	.0240
.0458	—	—	—	24.3	15.0	.0465
.0458	.025	.025	.025	24.5	15.5	.0450
.0915	—	—	—	33.0	15.0	.0900
.0915	.050	.050	.050	34.3	16.2	.0905

sideration; deeply colored samples contain two and three times as much tannin as light colored samples, and hence the size of the sample of the former products must be considerably reduced.

In the wine method the sample, after the removal of alcohol, is treated directly with carbon. Free acids are readily adsorbed on carbon,<sup>7</sup> in consequence of which the values are high. Tannin in neutral solution is quantitatively separated from other acids, thereby making the leaching of the adsorption complex unnecessary.

In the method here proposed calcium carbonate has been chosen as the neutralizing agent. Not only is neutralization with calcium carbonate more easily controlled but the insoluble calcium complex also acts as a clarifying agent, thereby greatly improving the sharpness of the titration end points.

In the wine method the oxidation value of tannin and coloring matter is given as .00416 gram of tannin per 1 ml. of .1N potassium permanganate solution. This value, it was found, does not conform to that for tannin; determinations on pure tannin (C<sub>14</sub>H<sub>10</sub>O<sub>9</sub>), analytical grade, gave a value of .0035.

<sup>7</sup> This Journal, 22, 357 (1939).

In the procedure here proposed .0035 has been accepted as the true oxidation factor of tannin, that is, 1 ml. of .1 *N* potassium permanganate solution is required to oxidize .0035 gram of tannin. In order to simplify the calculation a strength of potassium permanganate solution equivalent to .002 gram of tannin per 1 ml. was chosen. A solution of this strength is prepared by diluting 576 ml. of 0.1 *N* potassium permanganate solution to 1 liter.

The data given in Table 5 were obtained in the following manner:

The acids were dissolved in 350 ml. of water, .3 gram of powdered  $\text{CaCO}_3$  was added, and the mixture was heated to boiling. The mixture was cooled, and after .5 gram of filter-cel had been added, it was again made to 500 ml. and filtered; 200 ml. of the filtrate was subjected to the indigo- $\text{KMnO}_4$  titration (b). The remaining filtrate was shaken 5 minutes with 1 gram of carbon, allowed to stand 30 minutes, and then filtered through a large fluted paper, *the solution being poured back until bright*; 200 ml. of the filtrate was subjected to the indigo- $\text{KMnO}_4$  titration (c).

$$\frac{.002(b-c)}{.4} \text{ or } .005(b-c) = \text{gram of tannic acid.}$$

#### ADDENDUM

In the scheme of analysis presented no consideration was given to the nature or composition of the products, since the methods are applicable to all fruit products regardless of composition.

The kind and quantity of the acid ingredients in the different types of fruits vary widely. Apples, plums, prunes, cherries, and quinces contain malic acid almost exclusively; the acidity of citrus fruits is due almost wholly to citric acid; the grape contains tartaric and malic acids; the acidity of the blackberry and its hybrids is made up of isocitric and malic acids. Tannic acid is a normal constituent of all fruits.

It is obvious that in the analysis of certain fruits some of the provisions made in the proposed scheme of analysis may be omitted in order to save labor and time. With the exception of the grape and the blackberry, natural fruits contain neither tartaric nor isocitric acid; therefore in these fruits malic acid may be determined directly on the isolated acid solution. On the other hand, since the composition of commercial fruit products (jams, jellies, juices) is unknown, short cuts cannot be taken in their analysis. Slightly, or amber, colored commercial products unless bleached do not contain isocitric acid; in these the isolated acid solution after the removal of tartaric acid may be subjected directly to polarization for the determination of malic acid. Blackberry juice is frequently added to fruit products rather deficient in natural color so that part of their acidity may be due to isocitric acid. Hence in the examination of a colored product the analyst is obliged to treat the isolated acid solution as though it contained isocitric acid.

Natural fruits, colored or uncolored, do not contain inactive malic acid; commercial products may contain the acid through the addition of the synthetic commercial preparation. The determinations of tartaric and citric acids are made directly on the isolated acid solution. Tannic acid cannot be determined on the isolated acid solution; the determination must be made on the sample solution.

Succinic acid occurs in the common fruits only in negligible quantities; in fermented beverages (wines, ciders) the quantity may reach considerable proportions. For the determination of succinic acid in fermented beverages the reader is referred to the method described in "Laboratoriumsbuch für den Nahrungsmittelchemiker," by A. Beythien, page 327 (1939). The method is based on the resistance of the acid to potassium permanganate oxidation. The oxidized solution is extracted with ether, and the acid is determined as the silver salt. A method for the determination of oxalic acid has been omitted from the scheme of analysis presented here, since the acid occurs only in negligible quantities in the ordinary fruit products of the market.

## METHODS OF ANALYSIS FOR FRUITS AND FRUIT PRODUCTS

### 1

#### PREPARATION OF SAMPLE SOLUTION

Comminute samples containing insoluble solids (jams, preserves, etc.) as directed in *Methods of Analysis*, A.O.A.C., 1940, 335.

(a) *Jams, jellies, preserves, marmalades, butters*.—Weigh 50 grams of the sample into a 400 ml. beaker, add 150 ml. of water and boil mixture gently 1 hour, replacing at intervals the water lost by evaporation. Cool, transfer to a 250 ml. volumetric flask, dilute to mark, and filter through high-grade absorbent cotton.

(b) *Fresh fruits, dried fruits*.—Weigh 50 grams of fresh fruit or 10 grams of dried fruit into a 400 ml. beaker and proceed as directed in (a).

(c) *Beverages (juices, wines, ciders)*.—Weigh 50 grams of sample into a 250 ml. volumetric flask, dilute to mark, and filter through cotton. In case of concentrates use a smaller quantity of sample.

### 2

#### REMOVAL OF PECTIN

Pipet 200 ml. of the sample solution into a 400 ml. beaker and boil down to ca. 60 ml.; while the solution is still hot add 2 ml. of normal  $\text{HNO}_3$ ,<sup>1</sup> mix and pour into a 250 ml. volumetric flask. Rinse the beaker with 10 ml. of hot water and finally with alcohol.<sup>2</sup> Cool, and dilute to mark with alcohol at 20°. Pour the mixture onto a funnel lined with cotton and collect at least 220 ml. of filtrate. Toward the end filtration is slow; by gathering the ends of the cotton and squeezing the incased residue the desired quantity of filtrate is secured.

### 3

#### ISOLATION OF POLYBASIC ACIDS

(The "Isolated Acid Solution")

Determine the titer (t) of 10 ml. of the alcoholic filtrate obtained in 2, in terms of .1 N NaOH, using phenolphthalein indicator. Pipet 200 ml. of the alcoholic solution into a 400 ml. beaker, add "2t+2" ml. normal NaOH, and place on the steam bath 30 minutes.<sup>3</sup> Cool the mixture under the tap, add 5 ml. of normal acetic acid,<sup>4</sup> and rinse with alcohol into a centrifuge bottle (ca. 250 ml. capacity). Add

TABLE 6.—*Tartaric acid*.015T - .050 = *net* grams tartaric acid (x) in aliquot. $\frac{x}{.51}$  = grams tartaric acid in sample taken for analysis.

MATERIAL	SAMPLE TAKEN FOR ANALYSIS	ADDED TARTARIC ACID <sup>4</sup>	TITER (T) .1 N NaOH	TOTAL TARTARIC ACID IN ALIQUOT	NET TARTARIC ACID (x) IN ALIQUOT	ADDED TAR- TARIC ACID RETURNED
	<i>grams</i>	<i>gram</i>		<i>gram</i>	<i>gram</i>	<i>gram</i>
Acid Solution <sup>1</sup>	10 ml. soln	.010	3.7 3.6	.056 .054	.006 .004	.012 .008
	25 ml. soln	.025	4.2 4.3	.063 .065	.013 .015	.025 .029
	50 ml. soln	.050	5.0 5.0	.075 .075	.025 .025	.049 .049
	50	—	3.3	.050	—	—
	50	.063	5.4	.081	.031	.061
	50	.125	7.5	.113	.063	.124
Commercial	25	—	7.8	.117	.067	—
Concord	25	.050	9.5	.143	.093	.051
Grape Juice	25	.100	11.2	.168	.118	.100
Commercial	50	—	4.5	.068	.018	—
Blackberry	50	.108	8.3	.125	.075	.112
Preserve <sup>2</sup>	50	.245	12.7	.191	.141	.241

<sup>1</sup> The "Acid Solution" contains .200 gram each of tartaric, citric, malic, succinic, tannic, and oxalic acids made to 200 ml.<sup>2</sup> Prepared in laboratory.<sup>3</sup> Contained .070% tartaric acid, added in manufacture.<sup>4</sup> Does not include the .050 gram of tartaric acid added to every determination to promote precipitation.

"6t" gram of finely powdered lead acetate<sup>5</sup> and shake vigorously 5 minutes. Add .2 gram of filter-cel, fill the bottle with alcohol, and mix thoroughly. Centrifuge 5 minutes at ca. 1000 r.p.m., decant the supernatant liquor, and discard. To the lead salts add 50 ml. of 80% alcohol<sup>6</sup> and shake until the salts are *completely* dispersed.<sup>7</sup> Fill the bottle with 80% alcohol, mix thoroughly, and centrifuge. Discard the liquor<sup>8</sup> and repeat the centrifuging operation. Disperse the lead salts in 50 ml. of water, dilute to a volume of 150 ml., and saturate with H<sub>2</sub>S gas. Shake ca. 1 minute and rinse into a 250 ml. volumetric flask. Make to mark and filter through a large fluted filter, *pouring back until bright*.<sup>9</sup>

## 4

## TARTARIC ACID

Dilute 20 ml. of the isolated acid solution obtained in 3 with 50 ml. of water and boil a few minutes to expel H<sub>2</sub>S. Cool, and determine titer (t) in terms of .1 N NaOH.

Pipet 200 ml. solution into a 400 ml. beaker and boil down to ca. 20 ml. Cool, rinse into a tared 300 ml. glass-stoppered Erlenmeyer flask, and adjust with water to a weight of ca. 40 grams. Add .0940 gram of purest Rochelle salt (50 mg. tartaric acid)<sup>10</sup> and ".2t" gram of K-acetate.<sup>11</sup> Add 1 ml. of glacial acetic acid and 160 ml. of alcohol. Cool under tap, add a dozen medium-sized glass beads, and shake vigor-

ously 5 minutes. Place the mixture in the refrigerator for 15 minutes, shake ca. 1 minute, and again place in the refrigerator for 15 minutes. Shake ca. 1 minute and immediately, while still cold, filter on asbestos in a Gooch crucible. Wash the Erlenmeyer and crucible with ca. 100 ml. of cold alcohol and allow to remain under suction 5 minutes. Mark the filtrate in the suction flask "F"<sup>12</sup> and set aside.

With hot water rinse the crucible and flask into a beaker and determine the titer (T) in terms of .1 N NaOH.

.015T - .050<sup>13</sup> = net grams tartaric acid (x) in the aliquot.

$$\frac{x}{.512} = \text{grams acid in the sample taken for analysis.}$$

## 5

## CITRIC ACID (NORMAL)

Boil down 200 ml. of the isolated acid solution obtained in 3 to ca. 20 ml., rinse into a 300 ml. glass-stoppered Erlenmeyer flask, and adjust with water to a weight of ca. 40 grams. Add 2 grams of KBr and 5 ml. of conc. H<sub>2</sub>SO<sub>4</sub>, heat to ca. 50°, and allow mixture to stand 5 minutes. Add 20 ml. of 5% KMnO<sub>4</sub> solution from a pipet slowly (1 or 2 ml. at a time), swirling the flask a few seconds after each addition. Allow the mixture to stand undisturbed 5 minutes and cool to 15°. Add FeSO<sub>4</sub> solution (200 grams of salt made to 500 ml. + 5 ml. of conc. H<sub>2</sub>SO<sub>4</sub>) slowly with constant

TABLE 7.—*Citric acid*

.424P = grams anhydrous citric acid (x) in aliquot.

$$\frac{x}{.51} = \text{grams anhydrous citric acid in sample taken for analysis.}$$

MATERIAL	SAMPLE TAKEN FOR ANALYSIS	ADDED CITRIC ACID	PENTABROM- ACETONE (P)	CITRIC ACID (x) IN ALIUQUOT	ADDED CITRIC ACID RETURNED
	<i>grams</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>
Acid Solution <sup>1</sup>	5 ml. soln	.005	.0024	.001	.002
	10 ml. soln	.010	.0094	.004	.008
	25 ml. soln	.025	.0283	.012	.024
	50 ml. soln	.050	.0589	.025	.049
	150 ml. soln	.150	.1768	.075	.147
Apple Jelly <sup>2</sup>	50	—	—	—	—
	50	.061	.0731	.031	.061
	50	.122	.1485	.063	.124
Commercial Concord Grape Juice <sup>3</sup>	25	—	.0024	.001	—
	25	.063	.0802	.034	.065
	25	.098	.1226	.052	.100
Commercial Blackberry Preserve <sup>4</sup>	50	—	.0896	.038	—
	50	.063	.1627	.069	.061
	50	.122	.2358	.100	.122

<sup>1</sup> The "Acid Solution" contains .200 gram each of citric, tartaric, malic, succinic, tannic, and oxalic acids made to 200 ml.

<sup>2</sup> Prepared in laboratory.

<sup>3</sup> Contained .02% citric acid.

<sup>4</sup> Contained .15% citric acid, added in manufacture.

agitation until the mixture starts to clear up. Shake 1 minute, continue the addition of the iron solution until the  $\text{MnO}_2$  is dissolved, and add a few ml. in excess. Add 20 grams of anhydrous  $\text{Na}_2\text{SO}_4$ , again cool to  $15^\circ$ , and shake vigorously 5 minutes. Immediately, while still cold, collect the pentabromacetone on asbestos in a Gooch crucible and wash the residual precipitate from the flask with a portion of the filtrate. Finally wash the crucible with 50 ml. of cold water and allow the crucible to remain under suction a few minutes. Dry the crucible overnight in a  $\text{H}_2\text{SO}_4$  desiccator<sup>14</sup> and weigh. Designate the weight as "a." Remove the pentabromacetone from the crucible with alcohol followed by ether, filling the crucible 3 times with each solvent. Dry the crucible 10 minutes in the water oven, place in the desiccator 5 minutes, and weigh. Designate the weight as "b."

.424 (a - b)<sup>15</sup> = grams anhydrous citric acid (x) in the aliquot.

$\frac{x}{.512}$  = grams acid in the sample taken for analysis.

## 6

## LAEVO MALIC ACID

With a small quantity of alcohol rinse the filtrate "F" obtained in 4 into a 500 ml. Erlenmeyer flask and add .03 (10t - 2T + 6.6)<sup>16</sup> gram of lead acetate dissolved in a few ml. of water. (The t and T values are taken from 4.) Shake vigorously 5 minutes, add .3 gram of filter-cel, and mix thoroughly. Fill a centrifuge bottle with the mixture and centrifuge. Discard the supernatant liquor, add the remaining mixture, and rinse the flask with ca. 40 ml. of alcohol. Fill the centrifuge bottle with 80%

TABLE 8.—*Malic acid (laevo)*

.0185V° = grams malic acid (x) in aliquot.

$\frac{x}{.41}$  = grams malic acid in sample taken for analysis.

MATERIAL	SAMPLE TAKEN FOR ANALYSIS	ADDED MALIC ACID	POLARIZATION	MALIC ACID (x) IN ALIQOUT	ADDED MALIC ACID RETURNED
	<i>grams</i>	<i>gram</i>	<i>V°</i>	<i>gram</i>	<i>gram</i>
Acid Solution <sup>1</sup>	10 ml. soln	.010	— .20	.004	.010
	20 ml. soln	.020	— .45	.008	.020
	50 ml. soln	.050	— 1.15	.021	.051
	100 ml. soln	.100	— 2.25	.042	.102
Apple Jelly <sup>2</sup>	38	—	— 1.95	.036	—
	38	.049	— 3.05	.056	.049
	38	.098	— 4.10	.076	.098
Commercial Concord Grape Juice	25	—	— .40	.007	—
	25	.049	— 1.45	.027	.049
	25	.098	— 2.55	.047	.098
Commercial Blackberry Preserve	63	—	— .45	.008	—
	63	.025	— .95	.018	.024
	63	.049	— 1.45	.027	.046

<sup>1</sup>The "Acid Solution" contains .200 gram each of *malic*, citric, tartaric, succinic, tannic, and oxalic acids made to 200 ml.

<sup>2</sup>Prepared in laboratory.



alcohol, mix thoroughly, again centrifuge, and discard the liquor. Disperse the salts in 50 ml. of 80% alcohol and fill the bottle with 80% alcohol. Mix thoroughly, centrifuge, and discard the liquor. Repeat the centrifuging operation. *Completely* disperse the lead salts in water and dilute to a volume of ca. 150 ml. Saturate with  $H_2S$  gas, shake ca. 1 minute, and transfer to a 250 ml. volumetric flask. Make to mark and filter through a large fluted paper, *pouring back until bright*.

Boil down 200 ml. of filtrate to ca. 10 ml. and with 20 ml. of water rinse into an accurately tared 50 ml. Erlenmeyer flask. Cool under the tap, add ca. .05 gram of citric acid, and neutralize (phenolphthalein) with normal NaOH ("t"). To the neutralized solution add 1 ml. of glacial acetic acid and 1 gram of sodium acetate. Add ".25t" gram of lead acetate and with water adjust the mixture to a weight of 40 grams ( $\pm .2$  gram). Shake the mixture 5 minutes and allow to stand 10 minutes. Filter through a small fluted paper into a small Erlenmeyer flask and add 2 grams of uranyl acetate. Shake the mixture vigorously 5 minutes, add .5 gram of carbon,<sup>17</sup> again shake 3 minutes, and allow to stand 10 minutes, shaking occasionally. Filter through a small fluted paper into a 200 mm. polariscope tube, discarding the first few ml. of filtrate and *pouring back until bright*.

Polarize in  $V^\circ$  at  $20^\circ$ , using white light. Make at least 5 readings and take the average. If control for adjusting to standard temperature  $20^\circ$  is lacking, determine the temperature of the polariscope and prepare the solution of the uranium complex at this temperature. Make readings after allowing the tube to remain in the trough of the instrument 30 minutes.

.0185  $V^\circ$  = grams laevo malic acid (x) in the aliquot.

$$\frac{x}{.41} = \text{grams acid in the sample taken for analysis.}$$

## 7

## INACTIVE MALIC ACID

The acid is calculated from (a) the total hydrazine precipitate, and (b) the polarization of the laevo malic acid.

(a) *Total Hydrazine Precipitate*.—Treat the filtrate "F" obtained in 4, as directed in 6, up to and including "add .25t gram of lead acetate and with water adjust the mixture to a weight of 40 grams ( $\pm .2$  gram)."

Shake the mixture 5 minutes, allow to stand 10 minutes, add .5 gram of carbon, and shake 3 minutes. Filter into a 50 ml. flask, *pouring back until bright*. *Drain thoroughly*.

Pipet 10 ml. of the filtrate into a 300 ml. glass-stoppered Erlenmeyer flask and set the remaining filtrate aside for the "Malic Acid Polarization."

To the 10 ml. filtrate in the Erlenmeyer flask add ca. 30 ml. of water and .05 gram of citric acid. Add .5 gram of KBr and 3 ml. of conc.  $H_2SO_4$ , heat to ca.  $50^\circ$ , and allow to stand 5 minutes. Add 10 ml. of 5%  $KMnO_4$  solution from a pipet slowly (1 to 2 ml. at a time), swirling the flask a few seconds after each addition. Allow to stand undisturbed 5 minutes and cool to  $15^\circ$ . Add 3%  $Na_2SO_3$  solution *dropwise toward the end*, until the  $MnO_2$  is just dissolved. As the  $MnO_2$  dissolves slowly, avoid a large excess. Now add the  $KMnO_4$  solution until a slight but distinct brownish precipitate of  $MnO_2$  is produced. Cool to  $15^\circ$ , add 20 grams of anhydrous  $Na_2SO_4$  and shake vigorously 5 minutes. Filter on asbestos in a Gooch crucible and wash with ca. 70 ml. of cold water. Rinse the filtrate into a 400 ml. beaker, and add the  $Na_2SO_3$  solution dropwise until the solution is decolorized. Add 15 ml. of HCl, mix thoroughly, and add immediately dinitrophenylhydrazine solution (.5 gram of 2,4-dinitrophenylhydrazine + 20 ml. of water + 10 ml. of conc. HCl, heated to boiling and filtered). Heat to boiling and place on the steam bath for 30 minutes, stirring at frequent intervals. Immediately, while still hot, filter on asbestos in a weighed Gooch crucible,

TABLE 9.—*Inactive malic acid*

3.28P—.0185V°=grams inactive malic acid (x) in aliquot.

 $\frac{x}{.41}$ =grams inactive malic acid in sample taken for analysis.

ACIDS TAKEN FOR ANALYSIS								INACTIVE MALIC ACID (x) IN ALIQUOT	ACIDS RETURNED		
LAevo MALIC	IN- ACTIVE MALIC	TAR- TARIC	CITRIC	TANNIC	ISO- CITRIC	HYDRAZINE PRECIPITATE P	POLARI- ZATION		LAevo MALIC	IN- ACTIVE MALIC	TAR- TARIC
gram	gram	gram	gram	gram	gram	gram	V°	gram	gram	gram	gram
.060	.025	—	.050	—	—	.0102	-1.30	.0094	.059	.023	—
.060	.050	—	.050	—	—	.0135	-1.30	.0202	.059	.049	—
.060	.125	—	.050	—	—	.0235	-1.35	.0521	.061	.128	—
.050	.050	.050	.100	.050	.030	.0130	-1.15	.0214	.053	.052	.052
.050	.100	.030	—	.050	.080	.0191	-1.10	.0420	.050	.103	.031

and wash with 100 ml. of hot water acidulated with HCl (100 water+5 HCl). Dry the crucible 30 minutes in the water oven and weigh. Designate the weight as "P."

(b) *Malic Acid Polarization*.—To the remainder of the filtrate in the 50 ml. flask add 2 grams of uranyl acetate and shake vigorously 5 minutes. Add .1 gram of filter-cel, mix thoroughly, and filter into a dry 200 mm. polariscope tube, *pouring back until bright*. Polarize in V° at 20°, using white light. Designate the reading as V°.

3.28 P—.0185 V°<sub>25</sub>=grams inactive malic acid (x) in the aliquot. $\frac{x}{.41}$ =grams acid in the sample taken for analysis.

8

## ISOCITRIC ACID

The acid is calculated from (a) the combined optical rotation of isocitric and laevo malic acids, and (b) the optical rotation of laevo malic acid.

TABLE 10.—*Isocitric acid*

.03(a-1.19b)=grams isocitric acid (x) in aliquot.

 $\frac{x}{.41}$ =grams isocitric acid in sample taken for analysis.

MATERIAL	SAMPLE TAKEN FOR ANALYSIS	ADDED ISOCITRIC ACID	POLARIZATIONS V°			ISOCITRIC ACID (x) IN ALIQUOT	ADDED ISO- CITRIC ACID RETURNED
			a	b	1.19b		
Commercial	grams	gram				gram	gram
Apple	50	.026	-2.95	-2.80	-3.33	.011	.027
Jelly	50	.052	-2.60	-2.75	-3.27	.020	.049
	50	.128	-1.70	-2.80	-3.33	.049	.120
Commercial	20	—	-1.15	-1.00	-1.19	—	—
Concord	20	.029	— .85	-1.05	-1.25	.012	.029
Grape Juice	20	.115	+ .30	-1.00	-1.19	.045	.110
Commercial	50	—	+1.20	— .45	— .54	.052	—
Blackberry Preserve*	50	.102	+2.70	— .45	— .54	.097	.110

\* Contains normally .254% isocitric acid.

(a) *Combined Optical Rotation of the Acids*.—Treat the filtrate "F" obtained in 4 as directed in 6 up to and including "Saturate with  $\text{H}_2\text{S}$  gas, shake ca. 1 minute, and transfer to a 250 ml. volumetric flask. Make to mark and filter through a large fluted paper, pouring back until bright."

Pipet 200 ml. of filtrate into a 400 ml. beaker and boil 5 minutes to expel  $\text{H}_2\text{S}$ . Neutralize with normal  $\text{NaOH}$  (phenolphthalein) and add .5 ml. of the alkali in excess. Boil down to ca. 10 ml. and with 20 ml. of water rinse into an accurately tared 50 ml. Erlenmeyer flask. Add 5 drops of glacial acetic acid and with water adjust to a weight of 40 grams ( $\pm .2$  gram). Add 2 grams of uranyl acetate and shake vigorously 5 minutes. Add .5 gram of carbon, shake 3 minutes, and allow to stand 10 minutes, shaking occasionally. Filter through a small fluted paper into a 200 mm. polariscope tube, pouring back until bright. Polarize in  $\text{V}^\circ$  at  $20^\circ$ , using white light. Designate the reading as "a."

(b) *Optical Rotation of Malic Acid*.—Determine the rotation of malic acid as directed in 6. Designate the  $\text{V}^\circ$  as "b."

.03 (a - 1.19 b)<sup>19</sup> = grams isocitric acid (x) in the aliquot.

$$\frac{x}{.41} = \text{grams acid in the sample taken for analysis.}$$

## 9

## TANNIN AND COLORING MATTER

(In terms of tannic acid— $\text{C}_{14}\text{H}_{10}\text{O}_8$ )

## REAGENTS

(a) *Standard potassium permanganate solution*.—Dilute 576 ml. of 0.1N  $\text{KMnO}_4$  solution to 1 liter. 1 ml. solution = .002 gram of tannic acid ( $\text{C}_{14}\text{H}_{10}\text{O}_8$ ).

(b) *Indigo solution*.—Dissolve 6 grams of sodium indigotin disulfonate in 500 ml. of water by heating; cool, add 50 ml. of conc.  $\text{H}_2\text{SO}_4$ , dilute to 1 liter, and filter.

TABLE 11.—Tannin and coloring matter

.002(b - c) = grams tannic acid (x) in aliquot.

$$\frac{x}{.32} = \text{grams tannic acid in sample taken for analysis.}$$

MATERIAL	SAMPLE TAKEN FOR ANALYSIS	ADDED TANNIC ACID	b $\text{KMnO}_4$	c $\text{KMnO}_4$	TANNIC ACID (x) IN ALIQUOT	ADDED TANNIC ACID RETURNED
	grams	gram	ml.	ml.	gram	gram
Commercial	50	—	17.9	16.4	.0030	—
Apple	50	.0286	22.4	16.4	.0120	.0281
Jelly	50	.0573	27.0	16.5	.0210	.0562
	50	.0856	31.8	16.7	.0302	.0850
Commercial	.20	—	24.5	16.2	.0166	—
Concord	20	.0286	29.6	16.9	.0254	.0275
Grape Juice	20	.0573	34.7	17.1	.0352	.0581
	20	.0856	39.1	17.3	.0436	.0844
Commercial	20	—	24.1	16.4	.0154	—
Blackberry	20	.0286	28.8	16.8	.0240	.0269
Preserve	20	.0573	34.3	17.3	.0340	.0581
	20	.0856	39.1	17.4	.0434	.0875

(c) *Carbon*.—Any one of the sensitized carbons on the market is suitable.

## 10

## DETERMINATION

Use 50 grams of a light colored product (apple, peach, pear, etc.) or 20 grams of a deeper colored product (blackberry, raspberry, strawberry, etc.). Boil down samples containing alcohol (fermented beverages) to remove alcohol.

Transfer the sample to a 500 ml. beaker, add 300 ml. of water, and boil gently 1 hour, replacing at intervals the water lost by evaporation. Cool, transfer to a 500 ml. volumetric flask, and dilute to mark. Mix thoroughly and filter through cotton.

Pipet 400 ml. of filtrate into a 600 ml. beaker, add .3 gram of powdered  $\text{CaCO}_3$ , and heat to boiling. Cool, transfer to a 500 ml. volumetric flask, and dilute to mark. Add .5 gram of filter-cel, mix thoroughly, and filter through a large fluted paper, *pouring back until bright*.

(a) Pipet 200 ml. of filtrate into a 2 liter porcelain dish, add ca. 800 ml. of tap water and *exactly* 20 ml. of the indigo solution. Add standard  $\text{KMnO}_4$  solution, 1 ml. at a time, stirring vigorously until the blue color changes to green, then add a few drops at a time until the color becomes golden yellow. Designate the ml. of  $\text{KMnO}_4$  solution required as "b."

(b) To the remaining filtrate add 1 gram of carbon, shake a few minutes, and let stand 30 minutes, shaking frequently. Filter through a large fast-flowing fluted paper, *pouring back until bright*. Pipet 200 ml. of filtrate into the porcelain dish, and add 800 ml. of tap water and 20 ml. of the indigo solution. Titrate with standard  $\text{KMnO}_4$  solution in precisely the same manner as described above. Designate the ml. of  $\text{KMnO}_4$  solution required as "c."

$.002(b - c)^{20} = \text{grams tannic acid (x) in the aliquot.}$

$\frac{x}{.32} = \text{grams acid in the sample taken for analysis.}$

*Notes on Methods of Analysis*

<sup>1</sup> The addition of the  $\text{HNO}_3$  is necessary to liberate acids.

<sup>2</sup> Alcohol unqualified is 95% strength.

<sup>3</sup> The treatment with alkali is for the purpose of saponifying esters.

<sup>4</sup> Acidification is necessary to keep the lead salts of the monobasic acids in solution.

<sup>5</sup> The quantity ".6t" is derived from  $.03t \left( \frac{200}{10} \right)$ . It expresses the grams of lead acetate required to form the lead salts of the acids contained in the 200 ml. solution. The grams of lead indicated is excessive by one-half and is generally sufficient. If an addition of lead to the supernatant liquor forms a further precipitate after 1 minute, add more lead acetate and repeat centrifuging.

<sup>6</sup> Dilute 80 ml. of 95% alcohol to 100 ml. with water.

<sup>7</sup> In order to effect *complete* dispersion of the salts it is advisable to shake first with a small quantity (50 ml.) of the alcohol before filling the bottle.

<sup>8</sup> The discarded liquor contains the lead salts of the monobasic acids and the sugars.

<sup>9</sup> The filtrate contains the polybasic acids, organic and inorganic.

<sup>10</sup> The addition of tartaric acid accelerates the precipitation of small quantities of tartaric acid.

<sup>11</sup> This quantity of potassium acetate furnishes an excess.

<sup>12</sup> With the exception of tartaric acid the filtrate "F" contains all the polybasic acids of the sample.

<sup>12</sup> In the formula ".015T—.050," .015T is the grams of total tartaric acid in the aliquot and .050 is the grams of added tartaric acid.

<sup>14</sup> The drying of the pentabromacetone can be expedited by aeration. Place the crucible in a drying train (H<sub>2</sub>SO<sub>4</sub> and soda lime) and aerate to constant weight.

<sup>15</sup> In the formula ".424(a-b)," .424 is the theoretical factor for converting pentabromacetone into anhydrous citric acid and "a-b" is the weight of pentabromacetone in the aliquot.

<sup>16</sup> The formula .03(10t-2T+6.6) expresses the grams of lead acetate required to precipitate the polybasic acids remaining in solution after the removal of the tartaric acid; 10t is the acidity of the 200 ml. isolated acid solution, 2T is the acidity of the tartaric acid removed, and 6.6 is the acidity of .050 gram of added tartaric acid, all in terms of .1 N alkali.

<sup>17</sup> Any one of the carbons offered on the market is suitable.

<sup>18</sup> The formula "3.28P—.0185V" is derived from

$$\frac{.0185}{.0156} \left( .712P \times \frac{40}{10.3} - .0156V^\circ \right),$$

in which .0185 is the polarization value under the conditions of analysis ("apparent") and .0156 is the polarization value of malic acid in pure solution, .712 is the hydrazine value of malic acid, P is the grams of hydrazine precipitate, and 10.3 is the average weight of the 10 ml. solution taken for the hydrazine precipitation.

<sup>19</sup> In the formula ".03(a-1.19b)," .03 is the rotation value of isocitric acid; "a" is the optical rotation of isocitric+malic acids under the conditions of analysis and "1.19b" is the correction for the rotation of the malic acid. Since there have been no occlusions of malic acid in the procedure, this correction is calculated by multiplying the V° (b) obtained in the malic acid determination itself (where there was

occlusion) by the fraction  $\frac{.0185}{.0156} = 1.19$ .

<sup>20</sup> In the formula ".002(b-c)," .002 is the grams of tannic acid (C<sub>14</sub>H<sub>10</sub>O<sub>6</sub>) per 1 ml. of .1 N KMnO<sub>4</sub> solution, "b" the ml. of KMnO<sub>4</sub> solution required in the direct titration, and "c" is the ml. of KMnO<sub>4</sub> solution used in the titration of the detannated solution.

## EXAMINATION OF OFNER'S METHOD FOR DETERMINATION OF INVERT SUGAR IN REFINED SUGARS

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The commercial white sugars produced from raw cane sugar by modern refinery methods or directly from the juices by the processes of beet sugar manufacture are of extremely high quality. Most samples of these sugars show a direct polarization in excess of 99.9°S., and therefore have but a minute quantity of residual impurity. However, even with material of such high purity, differentiation and classification are necessary, and the choice of delicate methods of analysis becomes important. One of the most significant impurities in these high-grade sugars is reducing sugar,

which in order of magnitude amounts usually to less than 0.04 per cent.

The official method of this Association for the determination of small percentages (less than 1.5 per cent) of invert sugar in the presence of sucrose is the one devised by A. Herzfeld.<sup>1</sup> This method has proved unsatisfactory for the analysis of high-grade sugar because the high concentration of caustic alkali causes a considerable hydrolysis of sucrose itself and thus the correction for its reducing action is many times greater than the quantity which it is desired to measure. Moreover, in spite of many efforts to improve the technic the sucrose correction has been found variable and uncertain.

In order to diminish the reducing action of sucrose, use has been made of copper reagents having lower alkalinity, and the copper-alkali carbonates have proved particularly useful for this purpose. But at even the lowest alkalinities that could be used feasibly, samples of purest sucrose showed some reducing power. Bates and Jackson,<sup>2</sup> however, by measuring the respective rates of reaction of sucrose and of invert sugar showed that the two reducing reactions could be sharply distinguished from each other and that sucrose free from invert sugar could be prepared by simple crystallization methods.

Among the modern methods for the analysis of refined sugars, four have received consideration, namely the visual methods devised by H. Main<sup>3</sup> and by H. C. S. de Whalley<sup>4</sup> and the titration methods of Spengler, Tödt, and Scheuer<sup>5</sup> and of Ofner.<sup>6</sup> Main's "Pot" method is capable of high accuracy but is too time-consuming for the analysis of any considerable number of samples. de Whalley's method is simple and convenient but until modified has too limited a range for general serviceability. Both of the titration methods were devised for the analysis of beet products, but they are serviceable for any other product of low invert sugar content. Preliminary experiments showed that the two methods yielded results of about the same precision. According to Spengler, Tödt, and Scheuer, 10 grams of pure sucrose yields reduced copper equivalent to 2 mg. of invert sugar. According to Ofner 10 grams of sucrose by his method reduces copper equivalent to 1 mg. of invert sugar; in the present examination of the method 1.1 mg. was found. There is but little choice between the two methods, but that of Ofner is slightly more convenient in that it specifies direct boiling instead of the use of a boiling water bath. Its range is slightly more restricted than that of the method of the German authors, but it is entirely adequate for the analysis of high-grade white sugars. Ofner's method was consequently studied in detail. This is the official method of the Czechoslovakian Republic.

<sup>1</sup> *Z. Ver. Rabensucker Ind.*, 35, 1002 (1885).

<sup>2</sup> *Bull. Bur. Standards*, 13, 82 (1916); S268.

<sup>3</sup> *Int. Sugar J.*, 34, 213, 460 (1932).

<sup>4</sup> *Ibid.*, 39, 300 (1937).

<sup>5</sup> *Z. Wirtschaftsgruppe Zuckerind.*, 86, 130, 322 (1936).

<sup>6</sup> *Z. Zuckerind. Czechoslovak Rep.*, 59, 52, 63 (1934).

## METHOD

## REAGENTS

*Copper solution.*—Dissolve 5.0 grams of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 10.0 grams of anhydrous  $\text{Na}_2\text{CO}_3$ , 300 grams of pulverized Rochelle salt, and 50 grams of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , or 19.8 grams of anhydrous salt in ca. 900 ml. of water, warming finally if necessary. When completely dissolved it is advisable to continue heating for 2 hours on the water bath to destroy mold spores. Cool, and adjust the volume to 1 liter. Treat with active carbon or kieselguhr and filter, or filter directly through sintered glass having fine pores. Preserve in a dark place.

*Sodium thiosulfate solution.*—Dissolve 4.00 grams of crystals and dilute to a volume of 500 ml. or, preferably, prepare a stock solution containing in 500 ml., 20.0 grams of crystals and 1 ml. of 1 *N* NaOH or 0.1 gram of  $\text{Na}_2\text{CO}_3$ . Dilute 100 ml. of this stock solution to 500 ml. as required. Standardize by titration against the following iodine solution.

*Iodine solution.*—Dissolve 2.05 grams of pure iodine in ca. 10 grams of iodate-free KI dissolved in a few ml. of water. Dilute to a volume of exactly 500 ml. and preserve in a dark place. This solution is 0.03230 *N*.

*Starch solution.*—Rub 2.5 grams of soluble starch and ca. 10 mg. of  $\text{HgI}_2$  in a little water. Dissolve in ca. 500 ml. of boiling water.

## PROCEDURE

Dissolve 20 grams of the sample in distilled water and dilute to 100 ml. Transfer 50 ml. of the solution containing not more than 20 mg. of invert sugar to a 300 ml. Erlenmeyer flask and add 50 ml. of the copper reagent. Mix well, add 50–100 mg. of pumice or talcum powder, and heat to boiling on an asbestos gauze plate in the course of 4–5 minutes. Judge the initial time of boiling, not when bubbles arise from the bottom of the flask, but when they burst at the surface in considerable number. Continue the boiling for exactly 5 minutes. Cool without agitation by immersion in cold water. Add 1 ml. of glacial acetic acid. Add with continuous agitation an accurately measured volume of iodine, 5–30 ml. according to the amount of copper reduced, being sure that an excess is finally present. Pour down the wall of the flask from a graduated cylinder 15 ml. of 1 *N* HCl. Stopper the flask and allow the iodine to react for ca. 2 minutes, occasionally agitating the solution. Titrate the excess of iodine with thiosulfate, adding starch as the end point is approached. Deduct the volume of the excess iodine from the volume added. If more than 5 grams of sucrose is present, each gram reduces copper equivalent to 0.11 ml. of iodine. Each mg. of invert sugar reduces copper equivalent to the volumes of iodine shown in Table 4.

The procedure here described is that of Ofner with but few minor modifications. Ofner prescribed the use of a wire gauze on which rested an asbestos card having a central hole 6.5 cm. in diameter. The flame thus played directly on the metal gauze, resulting in a “flame-spot,” which could but cause local superheating. In the modified directions an ordinary asbestos gauze plate is specified, which doubtless produces more uniform heating.

In the iodometric determination Ofner prescribed the addition of hydrochloric acid before the addition of the standard iodine. The resulting cuprous chloride in acid solution is rapidly oxidized by air, the amount of oxidation depending upon the delay in adding the iodine. In the modified procedure the solution is first acidified with acetic acid, then the

iodine is added, and finally the hydrochloric acid. In one typical instance a difference of 0.4 ml. of iodine was found between the two procedures. The expedient was tried of adding to the alkaline solution 0.0323 *N* potassium iodate-iodide, which releases the equivalent iodine only after acidification. The higher acidity required, however, caused the precipitation of acid potassium tartrate, which obscured the end point seriously.

In a systematic manner Ofner varied the composition of his copper reagent in an effort to secure greater sensitivity to invert sugar. By partial substitution of trisodium phosphate for disodium phosphate in 10 per cent gradations, thus gradually increasing the alkalinity, he found that the sensitivity increased from about 2.0 to a maximum of 2.7 mg. of copper per milligram of invert sugar. The gain in sensitivity is, however, offset by the increased reduction by 10 grams of sucrose, which increased from zero for pure disodium phosphate (plus 8.1 grams of sodium carbonate per liter) to 8.7 mg. for pure trisodium phosphate. Since the copper analysis is the most accurate step in the procedure Ofner wisely adopted a solution containing disodium phosphate. He, however, increased the alkalinity by the addition of 10 grams of sodium carbonate (instead of 8.1 grams). This results in a slight but accurately correctible reducing effect of sucrose.

Disodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) crystals are highly efflorescent. A stock sample (cork-stoppered bottle) was found to have but 8 molecules of water. Likewise a sample recrystallized and allowed to dry in air overnight had lost about 4 molecules of water. It was, therefore, important to determine how great a variation in its composition could be tolerated without appreciable effect on the analytical results.

A quantity of crystals was pulverized and analyzed for water with sufficient accuracy by drying at 100°C. overnight and igniting to tetrasodium pyrophosphate. Its weight multiplied by 2.6936 gives the equivalent weight of disodium phosphate dodecahydrate. The copper reagent was prepared as specified but with the addition of varying quantities of sodium phosphate expressed as percentage of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 100 per cent meaning 50 grams per liter. The results of the analyses of a sample of commercial white sugar are shown in Table 1.

The results in Table 1 show that considerable variation in sodium phosphate concentration causes but little change in the amount of reduced copper. For the higher grades of commercial granulated sugar, which usually contain but a few hundredths of a per cent of invert sugar, the variations of phosphate concentration have no appreciable effect. For the samples containing 10 and 20 mg. of invert sugar or, respectively, 0.1 and 0.2 per cent of a 10 gram sample, differences in iodine consumed occur. If it is assumed that these differences are due to analytical error, the maximum deviation from the average is in no case greater than 0.2 ml., corresponding to an invert sugar content of 0.002 per cent of a 10 gram



TABLE 1.—*Effect of variation of concentration of sodium phosphate*

(Sucrose,<sup>1</sup> 10 grams. Sodium phosphate expressed as percentage of 50 grams of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  per liter. It is assumed that 50 grams of the salt of varying degrees of dehydration has been taken.)

SODIUM PHOSPHATE PERCENTAGES OF $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	MOLECULES OF CRYSTAL WATER	ML. OF 0.0323 N IODINE CONSUMED		
		NO INVERT SUGAR ADDED	INVERT SUGAR 10 MG.	INVERT SUGAR 20 MG.
100	12	2.1	12.7	22.7
115	9.4			23.0
125	8.0	2.2	12.9	23.1
130	7.4			22.9
150	5.4	2.15	13.0	22.8

<sup>1</sup> Commercial granulated sugar containing about 0.01% of invert sugar.

sample. This is the order of magnitude of the precision claimed for the method. It is considered desirable, however, to adhere to the specification that 50 grams of the dodecahydrate or 19.8 grams of anhydrous salt in 1 liter of copper reagent be taken.

A period of boiling of exactly 5 minutes is specified by the method. There is necessarily some uncertainty respecting the moment to select for the beginning of this period. While the talcum effectively prevents superheating, the actual beginning of the boiling is gradual. The moment was selected when bubbles of steam rose and burst at the surface in considerable number. This point, then, becomes a matter of individual judgment, and it is desirable to ascertain the effect of variations in selecting the initial point.

Two series of analyses were made, one with commercial white sugar containing about 0.012 per cent of invert sugar; the other the same sample with 10 mg. of invert sugar added. The results are shown in Table 2.

TABLE 2.—*Effect of variation of period of boiling*  
(Commercial white sugar containing 0.012% invert sugar)

TIME OF BOILING	IODINE, 0.0323 N CONSUMED	
	NO ADDED INVERT	10 MG. INVERT ADDED
	POUND	POUND
minutes	ml.	ml.
2	1.55	10.83
3	1.72	11.75
4	1.95	12.54
5	2.30	13.00
6	2.52	13.39
8.5	3.03	14.09

For the 5 minute period of boiling a half-minute variation in selecting the initial time causes a difference of 0.18 ml. of iodine for the granulated sugar ( $=0.0018$  per cent) for undertime and .11 ml. of iodine for over-time (.0011 per cent). For the granulated sugar plus 0.1 per cent added invert sugar a half-minute overtime causes a difference of 0.19 ml. ( $=0.0019$  per cent) and a half-minute undertime causes a deficiency of 0.23 ml. ( $=0.0023$  per cent).

Ofner stated that 10 grams of sucrose reduced copper equivalent to 1.0 ml. of iodine and thus that a correction of 0.1 ml. should be applied for each gram of sucrose taken. While this is approximately confirmed in the present study, slightly larger values are found for the higher concentrations of sugar, doubtless because of the slight modification of procedure. Diminishing values are found for the very low concentrations.

The results, expressed in terms of 0.0323 *N* iodine, are given in Table 3.

TABLE 3.—*Reducing action of pure sucrose*

SUCROSE	0.0323 <i>N</i> IODINE OBSERVED	0.0323 <i>N</i> IODINE FROM CURVE*	0.0323 <i>N</i> IODINE/GRAM SUCROSE
<i>grams</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
1	0.05	.05	.05
2	.16	.17	.09
3	.30	.29	.10
4	.42	.41	.10
5	.52	.52	.10
6	.66	.64	.11
7	.78	.76	.11
8	.86	.88	.11
9	.99	1.00	.11
10	1.11	1.11	.11

\* When the ml. of 0.0323 *N* iodine observed are plotted against the grams of sucrose present the points fall on either side of a smooth curve.

For the analysis of pure sucrose it seemed advisable to employ more dilute iodine and thiosulfate solutions. The two standard solutions were therefore diluted tenfold, corresponding to 0.00323 *N*, and standardized by means of a 0.0323 *N* potassium iodate solution (0.3721 gram of pure potassium iodate in 1 liter) in the following manner:

To 5 ml. of the iodate solution add 0.4 gram of KI and 10 ml. of 1 *N* H<sub>2</sub>SO<sub>4</sub>, and titrate the liberated iodine with the diluted thiosulfate solution in the usual manner. The diluted iodine solution is standardized against this thiosulfate solution.

Ofner's solution contains 63.65 mg. of copper in 50 ml. Since the ratio of reduced copper to invert sugar is about 2, it should be possible to analyze samples containing a maximum of about 30 mg. of invert sugar. It will be shown later in this paper that it is advisable to take samples containing not more than 20 mg. In a 10 gram sample 20 mg. amounts

TABLE 4.—*Analysis of sucrose-invert sugar mixtures*

SUCROSE	ML. OF 0.0323 N IODINE REDUCED			SUCROSE	ML. OF 0.0323 N IODINE REDUCED		
	TOTAL VOLUME	BY SUCROSE (TABLE 3)	BY INVERT SUGAR		TOTAL VOLUME	BY SUCROSE (TABLE 3)	BY INVERT SUGAR
5 mg. of invert sugar				15 mg. of invert sugar			
1	5.20	.05	5.15	1	15.58	.05	15.53
1	5.18	.05	5.13	1	15.71	.05	15.60
2	5.78	.17	5.61	2	16.19	.17	16.02
2	5.43	.17	5.26	2	15.71	.17	16.54
5	5.80	.52	5.28	5	16.33	.52	15.81
5	5.62	.52	5.10	5	16.16	.52	15.64
10	6.72	1.11	5.61	10	16.92	1.11	15.81
10	6.51	1.11	5.40	10	16.91	1.11	15.80
10	6.27	1.11	5.16	10	16.96	1.11	15.85
		Av.	5.300			Av.	15.740
1 mg. of invert requires 1.060 ml. of 0.0322 N iodine				1 mg. of invert requires 1.049 ml. of 0.0323 N iodine			
10 mg. of invert sugar				20 mg. of invert sugar			
1	10.52	.05	10.47	1	20.75	.05	20.70
1	10.46	.05	10.41	1	20.96	.05	20.91
2	11.04	.17	10.87	2	21.12	.17	20.95
2	10.55	.17	10.38	2	20.96	.17	20.79
5	11.06	.52	10.54	5	21.36	.52	20.84
5	11.06	.52	10.54	5	21.36	.52	20.84
10	11.76	1.11	10.65	10	21.88	1.11	20.77
10	11.75	1.11	10.64	10	21.85	1.11	20.74
10	11.76	1.11	10.65	10	22.11	1.11	21.00
		Av.	10.572			Av.	20.838
1 mg. of invert requires 1.057 ml. of 0.0323 N iodine				1 mg. of invert requires 1.042 ml. of 0.0323 N iodine			
				25 mg. of invert sugar			
				1	25.53	.05	25.48
				1	25.32	.05	25.27
				2	25.68	.17	25.51
				2	25.84	.17	25.67
				5	26.19	.52	25.67
				5	26.02	.52	25.50
				10	26.59	1.11	25.48
				10	26.39	1.11	25.28
				10	26.51	1.11	25.40
						Av.	25.473
				1 mg. of invert requires 1.019 ml. of 0.0323 N iodine			

TABLE 5.—0.0323 *N* iodine reduced by sucrose-invert sugar mixtures

Grams of sucrose

INVERT SUGAR	1	2	3	4	5	6	7	8	9	10
0	.05	.17	.29	.41	.52	.64	.76	.88	1.00	1.11
5	5.35	5.47	5.59	5.71	5.82	5.94	6.06	6.18	6.30	6.41
10	10.62	10.74	10.86	10.98	11.09	11.21	11.33	11.45	11.57	11.68
15	15.79	15.91	16.03	16.15	16.26	16.38	16.50	16.62	16.74	16.85
20	20.89	21.01	21.13	21.25	21.36	21.48	21.60	21.72	21.84	21.95
25	25.52	25.64	25.76	25.88	25.99	26.11	26.23	26.35	26.47	26.58

The values for zero invert sugar are taken from Table 3. The values for 5, 10, 15, 20, 25 mg. of invert sugar are from Table 4.

to 0.20 per cent; Ofner recommends 15 mg. as a maximum amount of invert sugar. The range can be extended if necessary by taking samples of 5, 2, or 1 grams and thus can include sugars containing up to 2.0 per cent of invert sugar. The range can of course be extended even to 100 per cent invert sugar by taking small enough samples, but for such materials other methods of analysis are more suitable.

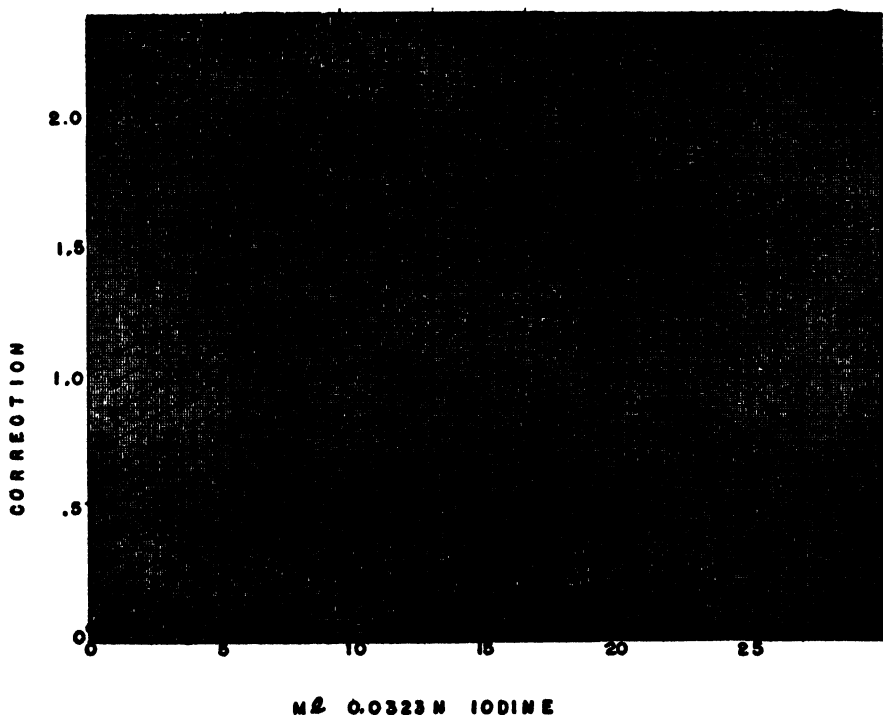


FIG. 1.—Corrections to be applied when converting iodine titer to milligrams of invert sugar. Figures on curves represent the amount of sucrose present.

TABLE 6.—Corrections to be applied to iodine titer in order to obtain mg. of invert sugar

ML. OF 0.0323 N IODINE SOLUTION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Sucrose	CORRECTIONS (To be subtracted)																								
grams	(1.11)	1.16	1.22	1.28	1.33	1.39	1.44	1.50	1.55	1.60	1.65	1.69	1.72	1.76	1.79	1.82	1.85	1.88	1.90	1.92	1.94	1.96	1.98	1.99	1.77
10	1.00	1.06	1.12	1.18	1.24	1.29	1.34	1.39	1.44	1.49	1.54	1.58	1.62	1.65	1.69	1.72	1.74	1.77	1.79	1.81	1.83	1.84	1.86	1.87	1.65
9	.89	.95	1.01	1.07	1.12	1.18	1.23	1.28	1.33	1.37	1.42	1.47	1.51	1.54	1.57	1.60	1.63	1.66	1.68	1.70	1.71	1.71	1.68	1.63	1.64
8	.77	.84	.90	.95	1.01	1.06	1.11	1.16	1.21	1.26	1.31	1.35	1.39	1.42	1.45	1.48	1.51	1.54	1.56	1.58	1.59	1.59	1.56	1.50	1.40
7	.66	.72	.78	.84	.90	.96	1.00	1.05	1.10	1.15	1.20	1.24	1.27	1.31	1.34	1.37	1.40	1.42	1.45	1.48	1.48	1.47	1.43	1.37	1.26
6	.55	.61	.67	.73	.78	.83	.88	.94	.99	1.03	1.08	1.12	1.16	1.19	1.22	1.25	1.28	1.30	1.32	1.34	1.35	1.34	1.30	1.23	1.11
5	.45	.51	.57	.62	.68	.73	.78	.83	.88	.93	.98	1.02	1.06	1.09	1.12	1.15	1.18	1.20	1.22	1.24	1.25	1.23	1.18	1.10	1.00
4	.34	.40	.45	.51	.56	.61	.67	.72	.76	.82	.86	.90	.93	.97	1.00	1.03	1.05	1.08	1.10	1.11	1.13	1.11	1.06	.98	.86
3	.22	.28	.34	.39	.45	.50	.55	.60	.65	.70	.75	.78	.82	.85	.88	.91	.94	.96	.98	1.00	1.01	.98	.93	.84	.72
2	.11	.17	.22	.28	.33	.39	.44	.49	.54	.59	.63	.67	.70	.74	.77	.80	.82	.84	.86	.88	.89	.86	.80	.71	.59

Example: A sample is known to contain 8 grams of sucrose. When analysed for invert sugar according to the procedure described in this paper 16.33 ml. of 0.0323 N iodine is consumed. From Table 6 a correction of 1.61 ml. must be subtracted from the ml. of iodine consumed in order to obtain the mg. of invert sugar present.  
 $16.33 - 1.61 = 14.72$  mg. of invert sugar.

Ofner for convenience of calculation recommends a deduction of 0.1 ml. of iodine for each gram of sucrose and the assumption that 1 ml. of iodine is equivalent to 1 mg. of invert sugar, although his experimental work did not show a constant ratio of iodine to invert sugar.

In order to determine the factors for conversion of iodine consumed to invert sugar, systematic analyses were made of samples containing 5–25 mg. of invert sugar and, respectively, 10, 5, 2, or 1 gram of pure sucrose. The results of these analyses are given in Table 4.

TABLE 7.—*Analysis of sucrose-invert sugar mixtures*

1	2	3	4	5	6	7	8	9
SUCROSE	INVERT-SUGAR	.0323 N IODINE	CORRECTION FROM TABLE 6	INVERT-SUGAR FOUND	ERROR COLUMN 5 MINUS COLUMN 2	INVERT SUGAR PRESENT	INVERT SUGAR FOUND	ERROR COLUMN 8 MINUS COLUMN 7
grams	mg.	ml.	ml.	mg.		per cent	per cent	per cent
10	15	16.69	1.84	14.9	-.1	.150	.149	-.001
9	15	16.67	1.73	14.9	-.1	.166	.165	-.001
8	15	16.52	1.62	14.9	-.1	.187	.186	-.001
5	15	16.20	1.26	14.9	-.1	.299	.297	-.002
3	15	15.99	1.03	15.0	0	.498	.498	0
2	15	15.87	.91	15.0	0	.744	.744	0
1	15	15.89	.79	15.1	+.1	1.478	1.487	+.009
10	10	11.78	1.68	10.1	+.1	.100	.101	+.001
9	10	11.64	1.57	10.1	+.1	.111	.112	+.001
8	10	11.52	1.45	10.1	+.1	.125	.126	+.001
7	10	11.32	1.33	10.0	0	.143	.143	0
6	10	11.39	1.22	10.2	+.2	.166	.175	+.004
5	10	11.28	1.09	10.2	+.2	.200	.204	+.004
4	10	11.24	.99	10.2	+.2	.249	.254	+.005
3	10	10.98	.87	10.1	+.1	.332	.332	+.004
10	4	5.19	1.34	3.8	-.2	.040	.038	-.002
10	4	5.22	1.34	3.9	-.1	.040	.039	-.001
8	2	2.87	1.00	1.9	-.1	.025	.024	-.001
8	2	2.92	1.01	1.9	-.1	.025	.024	-.001

From these experimental data, together with the values for sucrose, Table 5 has been compiled, and a graphic representation of the corrections to be applied to the volume of standard iodine reduced has been prepared (Figure 1). The solid curves represent the experimental work; the remaining curves are calculated.

It may be readily seen that the rapid decrease in the correction factor when more than 20 mg. of invert sugar is present makes it advisable to have not more than this amount of invert sugar in the sample to be analyzed.

Table 6 lists the correction to be applied to the iodine titer when various amounts of sucrose are present. These values were taken from Figure 1.

In order to further study the applicability of the method a series of analyses was made in which both the sucrose and invert sugar content was varied considerably. In calculating the results the correction factors given in Table 6 were used. The results of these analyses are given in Table 7.

Further study is contemplated in the range of 10 grams of sucrose and 0-5 mg. of invert sugar.

The experiments described in this paper constitute a confirmation of the validity of Ofner's method for the examination of refined sugars. It is incomparably more reliable and more nearly accurate than the Herzfeld method, which it is intended to replace. Values slightly different from those of Ofner were found for the sucrose corrections and slightly different factors for the conversion of the iodine titer to invert sugar. Even these differences are inconsiderable. Thus, if an iodine titer of 2.2 ml. is assumed for 10 grams of sugar, the present system of corrections and factors would yield a value of 0.010 per cent, while Ofner's values would yield 0.012 per cent. For an iodine titer of 11.8 ml. the respective yields would be 0.101 and 0.108 per cent, and for a titer of 21.8 ml., 0.198 and 0.208 per cent. The question remains whether the refinements here suggested are valid only in a single laboratory by one group of analysts or whether they possess general validity. This question can best be answered by the system of collaborative analytical work which is the practice of this Association.

### RAPID MODIFICATION OF A.O.A.C. CHLOROPLATINATE METHOD FOR DETERMINATION OF POTASSIUM IN FRUIT PRODUCTS

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The time-consuming steps in the present A.O.A.C. chloroplatinate method<sup>1</sup> include chiefly the oxalate treatment with subsequent sulfating and ignition. Both Gerritz<sup>2</sup> and St. John<sup>3</sup> have published procedures that omit the addition of oxalate in the determination of potassium. Good agreement on apple juice was obtained by St. John with the official chloroplatinate method for potassium in plants<sup>4</sup> and his more rapid method. Therefore, it occurred to the writer that the oxalate treatment and subsequent removal of ammonium salts, although possibly necessary in certain more complex products, may be dispensed with in the case of the ash obtained from fruits and preserves.

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<sup>1</sup> *This Journal*, 25, 91 (1942).

<sup>2</sup> *Ibid.*, 232.

<sup>3</sup> *Ind. Eng. Chem., Anal. Ed.*, 14, 301 (1942).

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1940, 130.

The major constituent in fruit ash, potassium, is associated with relatively small quantities of calcium and magnesium and with only traces of other metallic constituents. It has long been known<sup>5</sup> that the chloroplatinates of calcium and magnesium, as well as of sodium, are fairly soluble in the 80 per cent alcohol used for washing the precipitated chloroplatinate in the Lindo-Gladding method. In addition, the ammonium chloride-saturated potassium chloroplatinate solution dissolves any additional salts that may precipitate in the strong alcohol solution used in the method. Thus, it appears reasonable that the rather large quantities of 90 per cent alcohol wash used in the fruit method would remove the small amounts of calcium and magnesium in addition to any sodium that would be encountered in the fruit ash. Since the weight of the potassium chloroplatinate is customarily obtained by difference between the weight of the crucible plus precipitate and tare of the crucible after the potassium salt has been washed out with hot water, any insoluble matter in the ash, such as silica, would not interfere in the estimation. The estimation of potassium, then, could be made directly on the ash simply by addition of excess of chloroplatinic acid, washing with 90 per cent alcohol and saturated ammonium chloride-potassium chloroplatinate solution, and weighing the precipitate soluble in hot water.

The details of the short procedure are as follows:

Add ca. 20 ml. of distilled water to ash obtained in the fruit analysis, acidify with HCl, and add ca. 3 drops excess. Mix, and scrub bottom and sides with policeman. Rinse policeman with distilled water. Proceed as directed in the A.O.A.C. method,<sup>6</sup> beginning "Add an excess of Pt solution. . . ."

#### EXPERIMENTAL

In order to establish the effect of calcium and magnesium, the following mixture was prepared:

K<sub>2</sub>O — 30 mg. added as KCl  
 CaO — 7.1 mg. added as CaCO<sub>3</sub>  
 MgO — 3.5 mg. added as Mg(NO<sub>3</sub>)<sub>2</sub>

The calcium and magnesium added represent more than is usually encountered in the fruit ash; 29.9 mg. of potassium oxide was recovered by the proposed method. St. John<sup>5</sup> has demonstrated recovery of even smaller amounts of potassium when mixed with much larger quantities of calcium. The solutions used for collaborative testing of the cobaltinitrite method and the A.O.A.C. chloroplatinate procedure in 1941 were run by the proposed method, and the results shown in Table 1 were obtained.

Other fruits and fruit products were analyzed for potassium by analysts in several laboratories\* by the proposed method and the official procedure.

<sup>5</sup> Scott, "Standard Methods of Chemical Analysis," pp. 869, 871. D. Van Nostrand Co. (1939).

<sup>6</sup> *This Journal*, 25, 434 (1942).

\* San Francisco, Chicago, and St. Louis stations, Food and Drug Administration.



TABLE 1.—*Results on potassium by proposed method and the A.O.A.C. chloroplatinate and cobaltinitrite methods*  
(K<sub>2</sub>O mg./100 grams of sample)  
Results obtained by 1941 collaborators<sup>1</sup>

IDENTIFICATION	PROPOSED SHORT METHOD	A.O.A.C. CHLORO- PLATINATE METHOD	A.O.A.C. COBALTI- NITRITE METHOD
Sample Solution A		Min. 411	408
Apricot fruit	(1) 421	Av. 417 (12)*	420 (12)*
100 ml.—15 grams	(2) 420	Max. 422	432
Solution B		Min. 89	91
Mixture of preserve ashes	(1) 96	Av. 95 (12)	97 (12)
20 ml. = 12 g. preserve	96	Max. 99	103
Juice 1—Currant	(1) 259	Min. 253	254
	(2) 259	Av. 257 (9)	259 (10)
		Max. 260	272
Juice 2—Raspberry	(1) 172	Min. 167	168
	(2) 171	Av. 170 (10)	172 (10)
		Max. 172	175
Juice 3—Pineapple	(1) 181	Min. 173	174
	(2) 180	Av. 178 (10)	179 (10)
		Max. 182	182

\* Number of determinations made.

Equally good results were obtained. Some of these data are given in Table 2 for comparison.

TABLE 2.—*Results obtained by other laboratories with proposed method*  
(K<sub>2</sub>O mg./100 grams of sample)

	PROPOSED SHORT METHOD	A.O.A.C. CHLORO- PLATINATE METHOD
Loganberry jam	104.4	102.4
Plum jam	117.7	116.3
Blackberry jelly	81.6	79.7
Black raspberry jelly	121.0	119.1
Plum fruit	146.6	145.1
Black raspberry juice	255.1	254.6
Blackberry juice	172.2	168.1
Grape juice	136.4	136.8
Pineapple	179.4	175.4
Blackberries	177.9	177.0
Blackberries	204.4	202.4

Analysts—H. Gerrits, R. A. Diek, and D. Williams.

<sup>1</sup> *This Journal*, 25, 429 (1942).

Some analysts may prefer to determine the potash by the proposed method volumetrically instead of gravimetrically, or may wish to use a titration for a check on the purity and composition of the weighed precipitate. A simple technic employing the aqueous solution obtained in dissolving the potassium chloroplatinate as specified in the method would involve little additional work. In fact, for a number of routine estimations, a titrimetric procedure may be less time consuming. Mitchell and Ford<sup>8</sup> used formic acid for the reduction of the platinum in an aqueous solution of the chloroplatinate precipitate. The weight of the platinum thus obtained was then used as a check on the composition of the compound. It was thought the Volhard titration might be used in the estimation of the potassium content of this solution by measurement of the chloride ions freed from the platinum complex. The following method was evolved for this volumetric determination.

#### REAGENTS

(In addition to those used in the gravimetric method)

- (1) *Formic acid*.—A.C.S. reagent grade.
- (2) *Nitric acid*.—50% by volume and 2% by volume. From A.C.S. reagent grade.
- (3) *Silver nitrate solution*.—Approximately .05 *N*. Standardize by the official method.<sup>9</sup>
- (4) *Ammonium thiocyanate*.—Approximately .05 *N*. Standardize against the above solution.

#### PROCEDURE

Follow the gravimetric estimation through the precipitation and washing. Dissolve the  $K_2PtCl_6$  by nearly filling a 30 ml. sintered-glass, medium porosity crucible, held in a bell jar filtering device, with boiling distilled water. Traces of alcohol will not interfere if the precipitate has not been previously dried. Stir the liquid with a small glass rod and then suck through into a beaker flask of 250 ml. capacity. Repeat until the platinum salt is completely dissolved (usually three fillings will suffice). Remove the flask, add ca. 1 ml. of the formic acid, heat just to boiling, and let simmer for ca. 2 minutes after the metallic Pt has formed. Add 10 ml. of the  $HNO_3$  mix, and add a small excess of the .05 *N*  $AgNO_3$ , accurately measured. Boil vigorously for 5 minutes, cool, and filter through a medium porosity, sintered-glass crucible. Wash the precipitate 5 or 6 times with 2%  $HNO_3$  solution, breaking up any lumps with a glass rod. Collect filtrate and washings in an Erlenmeyer flask. Add 5 ml. of saturated ferric alum and titrate with  $NH_4SCN$  with vigorous agitation to the first definite end point.

1 ml. of .05 *N*  $AgNO_3$  = .785 mg. of  $K_2O$  or .65 mg. of K.

(The  $AgCl$ -Pt mixture can be scraped from the Gooch and collected; when a suitable amount is available, it can be dissolved in ammonia and the Pt converted to chloroplatinic acid in the usual way.)

The volumetric method was tested on pure potassium chloroplatinate. The results shown in Table 3 were obtained.

<sup>8</sup> *Ind. Eng. Chem., Anal. Ed.*, 15, 56 (1943).

<sup>9</sup> *This Journal*, 24, 100 (1941).

TABLE 3.—*K<sub>2</sub>O recovery from known quantities of pure K<sub>2</sub>PtCl<sub>6</sub> by volumetric method*

K <sub>2</sub> O ADDED AS K <sub>2</sub> PtCl <sub>6</sub> :	K <sub>2</sub> O FOUND
mg.	mg.
3.88	3.81
9.71	9.70
19.41	19.45
20.00	20.00
29.12	29.20
39.29	39.28

Some of the precipitates obtained by the short gravimetric method were examined for potassium by the method presented here. The results are shown in Table 4.

TABLE 4.—*Gravimetric and volumetric recovery of K<sub>2</sub>O*

	SHORT GRAVIMETRIC METHOD	VOLUMETRIC METHOD
	mg.	mg.
Strawberry jelly	13.20	13.20
Raspberry jam	29.08	29.00
Raspberry juice	27.07	27.02
Raspberry juice	27.22	27.33
Blackberry fruit	31.12	31.30
Apricot fruit	45.51	45.36
Loganberry fruit	18.37	18.29
Apple cores and peels	14.98	14.96
Apple jelly	11.18	11.15
Apple jelly	11.12	11.10
Apples	18.52	18.50
Apples	18.45	18.46
Apple juice	18.31	18.35
Citrus pectin	19.26	19.37

### SUMMARY

A short gravimetric chloroplatinate method for the determination of potassium, herein applied to the analysis of fruit products, requires considerably less working time and analytical manipulation than does the A.O.A.C. method. Check analyses between the two methods indicate good agreement. A volumetric procedure for determination of the potassium chloroplatinate thus obtained is also suggested.

## NOTES

### Fenton Reaction for Tartrates\*

The Fenton color reaction for tartrates offers a generally specific and sensitive method for the detection of tartaric acid and its salts. Thus citric, malic, succinic, oxalic, and acetic acids give negative reactions. So do sugars. References to the reaction<sup>1,2</sup> often fail, however, to give adequately detailed, procedural directions. Certain conditions must be maintained or the test will lose its sensitivity, and may fail altogether.

Typical directions<sup>1</sup> state: "If to an aqueous solution of the acid or soluble tartrate, 1 drop of  $\text{FeSO}_4$  solution, a few drops of  $\text{H}_2\text{O}_2$  solution, and an excess of  $\text{NaOH}$  be added, a deep violet color is produced."

A limited investigation of the Fenton test has shown that the relative concentrations of ferrous sulfate and hydrogen peroxide are of great importance. The test is successful only if an excess of ferrous sulfate is present. If enough hydrogen peroxide is added to oxidize all the ferrous sulfate the test gives neither blue nor purple color. On the other hand the presence of excess iron obscures the color, iron hydroxide being precipitated when alkali is added.

Chlorides, phosphates, and great quantities of citrates interfere with the test, whereas the presence of sulfates and acetates does not.

The following revised directions have been used successfully for the detection of tartrates in cosmetics:

#### PROCEDURE

To 5 ml. of a solution of tartaric acid, or a tartrate acidified with acetic acid, add 1 drop of a 5% solution of  $\text{FeSO}_4$  and 1 drop of a 3% solution of  $\text{H}_2\text{O}_2$ . Mix and add 5 ml. of 10%  $\text{NaOH}$  (w/v).

In the presence of 0.1% or more of tartaric acid a purple color develops.

NOTE: The test may fail in the presence of chlorides, phosphates, and large quantities of citrates.

### Determination of Monochloroacetic Acid in Wines†

The method for the determination of monochloroacetic acid in beverages (*This Journal*, 25, 145) is applicable to non-alcoholic beverages only. When occasion arose to apply it to wines the alcohol was removed by distillation before the extraction of monochloroacetic acid. The first procedure used was as follows:

Distil 100–200 ml. of wine, collecting 2 ml. of distillate for each 1 ml. of alcohol present in the sample used. Cool the residue, dilute to 150 ml., add 2 ml. of  $\text{H}_2\text{SO}_4$ , and proceed as directed on page 147 (*loc. cit.*) beginning "Extract with ether for 2 hours." Correct for any loss of monochloroacetic acid in the alcohol distillation by adding to the distillate a sufficient quantity of 10 N  $\text{NaOH}$  to make it 1 N, hydrolyze by digesting for 2 hours on the steam bath, and determine  $\text{Cl}$  ion by the procedure recommended for quantities of monochloroacetic acid less than 10 mg. (see page 150, *loc. cit.*).

It has recently been found that the wine procedure can be facilitated without decrease of accuracy by dispensing with the distillation and postponing the elimination of alcohol until after the ether extraction. The procedure now recommended follows:

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\* By I. S. Shupe (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

<sup>1</sup> Mulliken, S. P., "Identification of Organic Compounds," 1st ed., Vol. 1, p. 48. (1905). John Wiley and Sons, New York.

<sup>2</sup> "Allen's Commercial Organic Analysis," 5th ed., Vol. 1, p. 712 (1905). P. Blakiston's Sons & Co., Philadelphia, Pa.

† By John B. Wilson (Food and Drug Administration, Federal Security Agency, Washington D. C.)

Place 100 ml. of wine in the extractor, add 50 ml. of water and 3 ml. of  $\text{H}_2\text{SO}_4$ , mix, and extract with ether for at least 3 hours. Tilt the extractor in such a way as to drain as much of the ether as possible into the flask; disconnect the flask, add ca. 10 ml. of 1 *N* NaOH, and rotate to extract the acid constituents into the lower layer. Add a small piece of red litmus paper. If the red color remains, add more NaOH until it turns blue. Add 25 ml. more of 1 *N* NaOH and rotate again. Evaporate on the steam bath, hastening evaporation by passing a current of air into the flask, until all of the ether and much of the alcohol are gone and the volume of liquid is reduced to 25 ml. or somewhat less. Digest on steam bath for 2 hours, or boil under a reflux condenser for 30 minutes. Add 50 ml. of water, 15 ml. of  $\text{HNO}_3$ , and a known volume of the standard  $\text{AgNO}_3$  solution (ca. 0.05 *N*) in excess. Shake 1 or 2 minutes; add ferric indicator and titrate the excess of Ag with the standard  $\text{NH}_4\text{CNS}$  solution. Titrate the quantity of  $\text{AgNO}_3$  solution equal to that used for the sample in the same way. The difference between the two titrations is a measure of the  $\text{CH}_3\text{ClCOOH}$ .

If the color of the solution is such as to obscure the end point, proceed as follows: Dilute to a definite volume, filter, and make the back titration on an aliquot of the filtrate; or, upon nearing the end point, filter through a folded filter, add  $\pm 50$  ml. of water to the flask when empty, and pour into the filter when it is empty. When all of the wash water has passed through the filter, complete the titration.

When 0.005 per cent or less of monochloroacetic acid is present, use weaker standard solutions obtained by diluting the standard  $\text{AgNO}_3$  and  $\text{NH}_4\text{CNS}$  at the rate of 20 ml. to 100 ml., producing solutions having a value of 1 ml. = 1 mg. of  $\text{CH}_3\text{ClCOOH}$ .

## BOOK REVIEWS

**Dictionary of Bio-Chemistry and Related Subjects.** By WILLIAM M. MALISOFF, Editor-in-chief, Professor of Biochemistry at the Polytechnic Institute of Brooklyn and 46 Collaborators. Philosophical Library, Inc., New York (1943). 579 pages. Price, \$7.50.

The purpose of this book is to provide readily accessible information upon words that are encountered by readers of biochemical literature. It is partly a glossary, containing definitions of conventional lengths, and partly an encyclopedia with short essays of the review type upon subjects of biochemical interest. Admittedly, it is an experimental effort which "had to be done within serious limitations of cooperation due to the outbreak of the war." The publishers announce a list of contributors who are outstanding investigators and reviewers in the biochemical sciences, but it appears that the talents of this distinguished group of scientists were not utilized to the extent that would have been desirable.

A disappointing feature of this work is the large number of cross references. The validity, propriety, and usefulness of these cross references are highly questionable. For example, on page 162 there are 19 cross references for terms related to creatine or creatinine in which the reader is instructed to "see creatine and creatinine metabolism." The review on creatine and creatinine metabolism is a meritorious discussion but the cross references to it do not contain any directions to page or paragraph numbers; hence, the reader, who presumably does not know the meaning of the term sought, is invited to search through the whole essay of 10 pages and use his own judgment as to what parts of the latter supply the definition he is seeking. Again, as a definition of nutrition, the reader is told to "see amino acids, physiology of." This is an odd teaching process: it implies that a large, fairly well outlined field of science may be defined in terms of one of its component branches—an attempt to define the whole in terms of one of its parts. The review on the "Physiology of the Amino acids" is an excellent one, but it does not give the student a satisfactory idea of what the word nutrition means. There is also an impressive lack of relationship between certain words and their cross references. For example, for a definition of "pain," the student is told to "see Wound Healing." Obviously, a dissertation on wound healing is not a definition of pain.

The reviewer did not examine this book in detail, as it is a dictionary, but random inspection brought to his attention many correct and well expressed definitions. However, certain definitions were found which, in the opinion of the reviewer, are incorrect and others were noted which are incomplete. The editors appear rather uncertain of the effects of alcohol where in defining whiskey they relate that this "liquid . . . is stated to have intoxicating effects when taken internally." Basal metabolism is well defined; however, on page 350 the editors give "basal metabolism" as the definition for "maintenance metabolism," with which the reviewer does not agree. Also, the reviewer is not convinced that "water metabolism" or "uremia" can be explained by following the editors' suggestion to "see creatine and creatinine metabolism."

A surprising lack of critical appreciation of biochemical science is exhibited in certain instances. To define "pancreatic activity test," the editors offer Winternitz's test, published in *Münch. med. Wochschr.* in 1913, a completely obsolete test, without apparent rationale. For "kidney insufficiency" the reader is told to "see Becher." Becher's Test for Kidney Insufficiency, published in *Deut. Arch. Klin. Med.*, in 1925, is without clinical or historical significance. Just what is to be accomplished by a non-critical reporting of obsolete chemical tests that never had clinical value or significance is not obvious to the reviewer.

The propriety of a part-glossary, part-encyclopedic type of work, to be con-

sidered a dictionary of biochemistry, is to be questioned. A lexicography of exact definitions of well selected biochemical terms would be of educational value. But in a rapidly changing science like biochemistry, reviews, even though they are meritorious and quite useful, represent a temporary aspect of the subject reported. The biochemical review or essay of today will be superseded by the review or essay of tomorrow. Thus, biochemistry is not readily adaptable to encyclopedic types of interpretation as the latter are expected to have a more enduring tenure of exact meaning. For these reasons, it seems to the reviewer that a glossary type of work containing well selected biochemical terms, correctly and concisely defined, would be the more useful type of dictionary for readers in the biochemical sciences.—JOSEPH H. ROE.

**Soil and Plant Analysis.** By C. S. PIPER, Waite Agricultural Research Institute, Adelaide, Australia. The Hassell Press, Adelaide, Australia. XIV+368 pp., illus. (1942). Price 15/- (Australian currency).

The book is well printed on good paper, illustrated with 19 line drawings. Those interested in soil and plant investigations should find this up-to-date monograph very helpful. It describes varied soil and plant analyses and tests in use at the Waite Institute. The literature cited is not extensive, but it is well chosen and adequate. The procedure for preparation of soil samples corresponds essentially with well established practice in the United States. No description is given of fundamental methods for the total analysis of a soil but a description of part of the fusion analysis procedure is given in a chapter entitled "Separation and Analysis of the Clay." There is included also the method of analysis of an extract obtained with hydrochloric acid of constant boiling point. In this connection the statement is made that digestion of soils with hydrochloric acid is now only occasionally used. Ion exchange is given the prominent place it deserves.

Various methods for determination of the hydrogen-ion concentration of soils are included. The statement is made that the glass electrode is probably the most nearly accurate method for determining *pH* in soils generally. In spite of the stated weakness of the quinhydrone method, its use is described in detail. A colorimetric method, useful to about one-half *pH* unit, is described, in which permanent color standards on porcelain are used for comparison.

Determinations of various constituents of soils are given in detail. A chapter is devoted to each of the following topics: Calcium carbonate, organic matter, free ferric oxide, nitrogen, nitrates, nitrites, and ammonia. About 70 pages are devoted to physical determinations and tests of soils. Under mechanical analysis are included the important methods in use throughout the world, together with supporting data that give a good idea of the importance of various factors involved. Methods for determining various single value constants of soils are described with pertinent comments. One particularly good suggestion is that from 3 to 5 minutes should be taken for bringing a moisture equivalent centrifuge to full speed. Inclusion of a chapter dealing with the determination of soil color is a particularly desirable feature.

Part II of the book is concerned with the inorganic composition of plants. Valuable suggestions are given for sampling and for preparation of plant materials for analysis, including a critical review of ashing methods. The directions for making determinations of the more common constituents follow essentially conventional lines of gravimetric or volumetric procedure. The treatment of minor or trace elements is given a prominent place and includes up-to-date procedures such as the one employing dithizone in carbon tetrachloride for the determination of zinc.

Some of the methods described will meet with little favor in the United States, but for the most part well recognized procedures are described, as well as many others that are valuable under particular circumstances.—M. S. ANDERSON.







DR. RICHARD FAY JACKSON, 1881-1943

## RICHARD FAY JACKSON

The members of the Association of Official Agricultural Chemists, who since 1919 had come to appreciate the painstaking reports of Dr. R. F. Jackson as Collaborator, Associate Referee, and Referee on Sugars and Sugar products, were grieved to learn of his untimely death on June 1, 1943. His last attendance at a meeting of the Association was on October 29, 1940. In May, 1941, he was stricken while playing golf with an attack of coronary thrombosis and, although recovering sufficiently before the end of the year to resume his work as Senior Chemist at the National Bureau of Standards, he did not fully regain his health. Increasing disability obliged him to give up all work in March of the present year, and his gradually declining strength did not enable him to withstand the final outcome.

Jackson was born in Boston, January 2, 1881, the son of Edward P. and Helen M. (Smith) Jackson. After attending the Boston Latin School, he received in 1903 his A.B. degree, *magna cum laude*, from Harvard, which later in 1917 conferred upon him the higher degree of Ph.D. Jackson began his professional career in 1907, when he was appointed Assistant Physicist at the National Bureau of Standards; he was promoted to Associate Chemist in 1917 and to Senior Chemist in 1922.

Jackson's researches on sugar analysis for the Association of Official Agricultural Chemists were conducted in the Polarimetric Section of the Bureau of Standards. In his active period of some thirty years he performed a number of important fundamental investigations, of which in the present sketch mention must be largely limited to those that concern the work of the Association.

In 1914 Jackson published an important paper on "Equilibrium in the System: Lead Acetate, Lead Oxide, Water at 25°,"<sup>1</sup> in which several long-existing uncertainties regarding the composition of the basic lead acetate reagent used in the clarification of sugar solutions were finally cleared away. This work was followed, in 1916, by the publication of an investigation, equally fundamental in character, on "The Saccharimetric Normal Weight and the Specific Rotation of d-Glucose,"<sup>2</sup> in which the normal weight of d-glucose, determined by a quartz wedge saccharimeter graduated for a normal weight of 26 grams of pure sucrose, was found experimentally to be 32.231 grams, which is very close to the theoretical value of 32.248 grams previously calculated by Browne.<sup>3</sup>

At the eighth meeting of the International Congress of Applied Chemistry in New York in 1912, F. J. Bates and R. F. Jackson called attention to a probable error in the graduation of quartz-wedge saccharimeters by the Herzfeld-Schönrock standard. Their continued work confirmed this first announcement, the final results appearing in 1916 under the title, "The Constants of the Quartz-Wedge Saccharimeter and the Specific Rotation of Sucrose. I. The Constants for the Twenty-six Gram Normal Weight,"<sup>4</sup> in which it was shown that the normal weight of 26 grams of pure sucrose gives a reading of only 99.89°, instead of 100.00°, as prescribed by the Herzfeld-Schönrock standard. This value, which was later confirmed by other investigators, is now accepted internationally for the graduation of saccharimeters employing a normal weight of 26 grams of sucrose.

In 1919 Jackson, in collaboration with C. L. Gillis, presented a paper at the 35th annual meeting of the Association on "The Double-Polarization Method for Estimation of Sucrose and the Evaluation of the Clerget Divisor,"<sup>5</sup> which in some of its four modified procedures has found use in many sugar-testing laboratories. At the 1923 meeting of the Association Jackson was appointed Associate Referee on Chemical Methods for Reducing Sugars, and reports upon this subject were given by him at consecutive meetings from 1924 to 1937, when he was appointed General

Referee. He presented reports in the latter capacity at the 1938, 1939, and 1940 meetings; his report for 1941 was not presented at the meeting but was later submitted for publication.

The work of Jackson as Associate and General Referee on Sugars and Sugar Products was characterized by great accuracy, careful attention to detail, and extreme caution in the making of recommendations. In the course of the Association's eventful history a confusingly large number of optional and tentative methods for determining reducing sugars had become incorporated in its *Methods of Analysis*, A.O.A.C. The correction of this situation was a part of the threefold aim which Jackson set before himself at the commencement of his long referee activities. It comprised "(1) incorporation of the results of recent research; (2) discarding duplicate or obsolete methods; (3) an examination of the limits of accuracy of the present methods and a scrutiny and verification of the present standard reference tables."<sup>8</sup> Jackson fully realized that the completion of such a program would require many years of laborious research. Although he did not live to fill in the full outline of what he hoped to accomplish he nevertheless introduced a considerable number of improvements in each one of the three fields which he mapped out for investigation.

Under the incorporation of the results of recent research, which Jackson effected in the methods of the Association, may be mentioned the adoption of the Scales method<sup>7</sup> for small amounts of reducing sugars, of the modified Lane-Eynon general volumetric method<sup>8</sup> for reducing sugars, of the Jackson-Mathews modification of Nyns' selective determination of levulose,<sup>9</sup> of a modification of the volumetric thio-sulfate method for determining reduced copper,<sup>10</sup> and of Somogyi's modification of Shaffer and Hartman's microchemical method.<sup>11</sup> Under the discarding of duplicate or obsolete methods Jackson recommended the deletion of the long published but little used Meissl method for invert sugar,<sup>12</sup> of the Wein method for maltose,<sup>13</sup> of the Soxhlet and Wein method for lactose,<sup>14</sup> of the ferrocyanide procedure<sup>15</sup> for testing the removal of copper in the volumetric determination of reducing sugars, and of the process of determining copper by reducing the precipitated cuprous oxide in hydrogen.<sup>16</sup>

In his examination of the limits of accuracy of certain official methods of the Association that had been long in use Jackson performed a most valuable service. At the 1933 meeting he presented a nine page report<sup>17</sup> on "A Critical Study of the Munson and Walker Method for Reducing Sugars." This work was continued and finally reported upon at the 1940 meeting by Jackson and Dr. Emma J. McDonald in their exhaustive twenty-one page report on "Errors of Munson and Walker's Reducing Sugar Tables and the Precision of Their Method,"<sup>18</sup> in which they recommended the determination of reduced copper by analysis instead of by direct weighing of cuprous oxide and the tentative adoption of Hammond's table, which if later made official, will be substituted<sup>19</sup> for the present official Munson and Walker's table.

Among other reports which Drs. Jackson and McDonald presented before the Association should be mentioned their papers on "The Basic Values of the Clerget Divisor and the Correction Coefficients"<sup>20</sup> and "The Normal Weight of Invert Sugar and a Test of Vosburgh's rule."<sup>21</sup>

While the present sketch is largely confined to Jackson's referee work for the Association, a passing mention should be made of the highly important work<sup>22</sup> which he and Dr. McDonald performed in isolating several new difructose anhydrides from the inulins of Jerusalem artichokes and dahlia tubers. He also, in association with C. G. Silsbee and M. J. Proffitt, devised a commercial process for manufacturing levulose from artichokes.<sup>23</sup> For his researches in this field he was awarded the first Hillebrand prize of the Washington Section of the American Chemical Society. Jackson was also one of the authors of Circular 440 of the National Bureau

of Standards, "Polarimetry, Saccharimetry and the Sugars," contributing especially to the sections relating to Chemical Methods for the Determination of Reducing Sugars, the Clerget Method and the Preparation and Determination of Levulose.

Those who were privileged to hear Jackson's impromptu referee presentations at the meetings of the Association had no conception of the great expenditures of time and energy that had been involved until his full reports were published in *The Journal*. Jackson was not a desk chemist. An uncertainty had to be subjected to rigid experimental proof and when a pressing problem engaged his attention he worked not only on holidays but frequently far into the night. Until his final breakdown Jackson enjoyed a number of outdoor recreations such as golf, automobile driving, and mountain climbing. In his younger years he was fond of high diving, and it was while indulging in this sport in the Tidal Basin of Washington that he incurred the ear infection that resulted in his partial deafness. He bore the physical afflictions of his last years with fortitude and resignation.

Dr. Jackson was a member of the American Chemical Society, the Washington Chemical Society, the Washington Academy of Sciences, and of the International Commission of Sugar Analysis. He was also a Fellow of the American Institute of Chemists. The Harvard Club and Cosmos Club of Washington and the Chevy Chase Country Club were among the social organizations to which he belonged. He was married on September 17, 1908, to Mabel Elizabeth Clark, to whom and to his daughter, Mrs. Carolyn Jackson Garrett, the sympathy of his many friends in the Association of Official Agricultural Chemists is extended.

C. A. BROWNE

<sup>1</sup> *J. Am. Chem. Soc.*, **36**, 2346-57

<sup>2</sup> *J. Wash. Acad. Sci.*, **6**, 530-531 (1916)

<sup>3</sup> *J. Ind. Eng. Chem.*, **2**, 526 (1910)

<sup>4</sup> *Bur. Standards Sci. Paper No. 268*, 67-128 (1916).

<sup>5</sup> *J. Assoc. Official Agr. Chem.*, **4**, 321 (1920-21); *Bur. Standards Sci. Paper No. 375* (1920)

<sup>6</sup> *J. Assoc. Official Agr. Chem.*, **8**, 402 (1925).

<sup>7</sup> *Ibid.*, **11**, 177 (1928)

<sup>8</sup> *Ibid.*, **9**, 35, 179 (1926)

<sup>9</sup> *Ibid.*, **15**, 79, 198-212 (1932)

<sup>10</sup> *Ibid.*, **18**, 83 (1935)

<sup>11</sup> *Ibid.*, **23**, 67, 559 (1940).

<sup>12</sup> *Ibid.*, **11**, 176 (1928).

<sup>13</sup> *J. Assoc. Official Agr. Chem.*, **22**, 61 (1939)

<sup>14</sup> *Ibid.*, **17**, 46, 170-171 (1934).

<sup>15</sup> *Ibid.*, 170.

<sup>16</sup> *Ibid.*, 295.

<sup>17</sup> *Ibid.*, 293-301.

<sup>18</sup> *Ibid.*, **24**, 767-788 (1941).

<sup>19</sup> *Ibid.*, **25**, 74, 674 (1942).

<sup>20</sup> *Ibid.*, **22**, 580-586 (1939).

<sup>21</sup> *Ibid.*, **25**, 675-680 (1942)

<sup>22</sup> *Bur. Standards J. Research*, **5**, 1151-60 (1930); **6**, 709-715 (1931)

<sup>23</sup> *Ind. Eng. Chem.*, **16**, 1250-51 (1924); *Bur. Standards Sci. Paper No. 519*, 587-617.



## REPORT ON HOPS

### WITH SPECIAL REFERENCE TO THE DETERMINATION OF THEIR ALPHA RESIN CONTENT

By FRANK RABAK (Bureau of Plant Industry, Soils and Agricultural  
Engineering, A.R.A., U. S. Department of Agriculture,  
Washington, D. C.), *Associate Referee*

In the scientific evaluation of the brewing quality of hops quantitative determination of their resinous constituents is necessary. Satisfactory methods<sup>1</sup> are now available for the accurate determination of the soft (alpha and beta) and hard (gamma) resins. The alpha resin, which is the most important brewing resin in hops, is usually determined gravimetrically by precipitation of the alpha lead salt from a methyl alcohol solution of the soft resins by means of a 1 per cent (by volume) solution of lead acetate in absolute methyl alcohol. In the adopted methods of analysis<sup>1</sup> two procedures are recommended for determination of the alpha resin content of hops, namely, (a) preliminary titration, by means of a spot test, of the methyl alcohol solution of the soft resins to determine the quantity of lead acetate solution necessary for complete precipitation of the alpha lead salt, and (b) rapid determination by direct addition of 7 ml. of lead acetate solution to precipitate the salt. In studies made by Ford and Tait<sup>2</sup> it is stated that the alpha lead salt, which is precipitated by the lead acetate solution, is somewhat soluble in excess of that reagent. In order to prevent addition of an excess of lead acetate solution it has been recommended that the preliminary spot test be used to determine the exact quantity of the solution required for complete precipitation of the alpha lead salt. This spot test is made either by allowing the precipitate of alpha lead salt to settle after each addition of the solution or by centrifuging the mixture successively in order to obtain a clear supernatant liquid for testing for excess of lead acetate solution. A drop of the clear liquid obtained after each addition of the solution is placed on a piece of filter paper alongside a drop of 10 per cent aqueous solution of sodium or ammonium sulfide until the margins interpenetrate. The end point of the reaction is reached when a brown coloration, indicating slight excess of lead acetate solution, is produced. The determination of the end point on which the exact quantity of lead acetate solution to be used is based requires considerable experience and the procedure consumes much time. Unless great care is exercised in testing a drop of the supernatant liquid it is possible that slight traces of the precipitated lead salt are apt to be transferred to the filter paper and thus give a false indication of an excess of the lead acetate solution. Unless sufficient reagent is used, incomplete precipitation will result. Definite information is lacking with

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<sup>1</sup> *This Journal*, 25, 292 (1942).

<sup>2</sup> *J. Inst. Brew.*, 38, 351 (1932).

TABLE 1.—Total soft resin content of hop samples and percentages of alpha resin as determined with and without spot test showing the effect of varying quantities of lead acetate solution used in their precipitation

ROD SAMPLE	TOTAL SOFT RESINS	WITH SPOT TEST		WITHOUT SPOT TEST										REDUCTION IN PERCENTAGE OF ALPHA RESIN CAUSED BY EXCESS OF LEAD ACETATE SOLUTION			
		ALPHA RESIN	LEAD ACETATE SOLUTION	VOLUME OF LEAD ACETATE SOLUTION USED FOR PRECIPITATION													
				3 ML.	4 ML.	5 ML.	6 ML.	7 ML.	8 ML.	9 ML.	10 ML.	1 ML.	2 ML.	3 ML.	4 ML.		
	per cent		ml.														
1	22.28	8.14	8.25	—	5.19	5.36	6.79	7.60	8.17	7.97	7.73	0.20	0.44	—	—		
2	18.14	6.50	7.25	—	4.61	5.50	6.51	6.62	6.33	6.03	5.71	0.29	0.59	0.91	—		
3	17.24	5.35	6.75	—	4.18	4.90	5.27	5.40	5.19	4.80	4.35	0.21	0.60	1.05	—		
4	15.60	4.78	6.50	—	4.26	4.57	4.95	4.61	4.26	3.96	3.68	0.34	0.69	0.99	1.27		
5	14.37	2.79	3.75	2.68	2.80	2.61	2.21	1.93	1.66	—	—	0.19	0.59	0.87	1.14		
6	13.18	1.74	3.50	1.71	1.80	1.51	1.05	0.89	0.69	—	—	0.29	0.75	0.91	1.11		

regard to the rate of precipitation and the solubility of the alpha lead salt in excess of the reagent.

In order to obtain such information and if possible to shorten the time required for the determination without undue sacrifice of accuracy, a study was made of the methyl alcohol solutions of the soft resins from six samples of hops, containing widely different percentages of both total soft and alpha resins, by using varying quantities of lead acetate solution for precipitation of the alpha lead salt. By this procedure it was possible to ascertain not only the exact quantity of reagent necessary for complete precipitation but also to what extent excess of this reagent causes noticeable solution of the precipitate with subsequent lowering of the readings of the alpha resin content. The samples were analyzed by the rapid determination and also by the spot test method. Table 1 lists the percentages of total soft resins in the six samples along with the readings of the alpha resin content in each at different stages of the precipitation and the reduction in percentage of the readings of this resin caused by excess lead acetate solution. Also included in the table, for comparison, are the percentages of alpha resin in each sample obtained by the spot test method and the quantity of lead acetate solution required for complete precipitation of their alpha lead salts by this method.

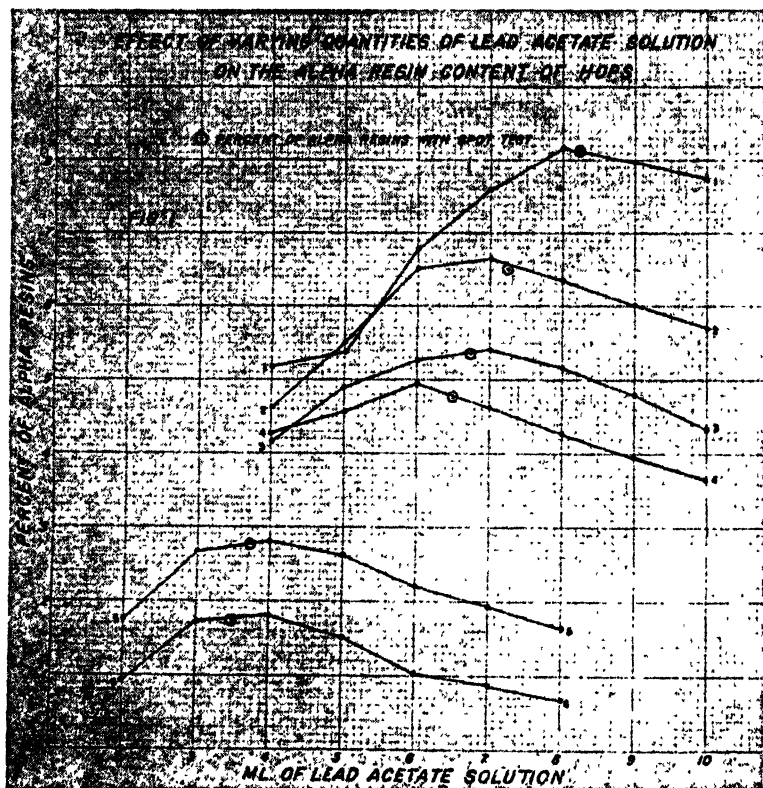
In obtaining the results on the alpha resin content shown in Table 1, aliquot portions (representing 1 gram of hops) of the methyl alcohol solutions of the soft resins of each sample of hops were used for the precipitation tests. The percentages of alpha resin determined by the spot test method are in all cases slightly lower but correspond very closely with those determined by direct addition of the lead acetate solution without the spot test. The quantities of lead acetate solution that were predetermined by the preliminary spot test and used for complete precipitation of the alpha lead salt also correspond fairly closely in most cases to the quantities of the reagent required for maximum precipitation without the spot test. With each succeeding milliliter of excess lead acetate solution used, a comparatively uniform reduction in readings of the alpha resin content resulted. The reductions due to the solubility of the alpha lead salt in 1 milliliter excess of the reagent are relatively small, ranging from 0.19 to 0.34 per cent, but with each milliliter of further excess they are noticeably greater.

Figure 1 shows graphically the comparatively uniform increase indicated in alpha resin content of the various hops with each succeeding addition of 1 ml. of the reagent used in the precipitation of the alpha lead salt. Also in every case, when the maximum percentages are reached, the reduction in percentages caused by excess of the reagent is remarkably uniform. The close correspondence of the percentages of alpha resin as determined by the spot test method with those obtained by direct addition of the lead acetate solution is noteworthy when the alpha resins range be-



tween 4.78 and 6.50 per cent. The results show that definite quantities of the lead acetate solution are required for complete precipitation of the alpha resin. Depending upon the alpha resin content of the particular samples of hops analyzed, from 4 to 8 ml. of lead acetate solution was required to completely precipitate the alpha lead salt from a methyl alcohol solution of the soft resins in one gram of hops.

As a result of tests conducted during three successive years by numerous



collaborators using both methods for the determination of the alpha resin content of hops, the American Society of Brewing Chemists approved and adopted the rapid method as an alternative procedure applicable to hops of average compositions, which comprise the bulk of commercially marketed hops. For such hops a fixed quantity of 7 ml. of lead acetate reagent has been found to give results that are fairly comparable with those obtained by the somewhat more nearly accurate but time-consuming spot test method.

Of the six samples used in the test, Nos. 2, 3, and 4 may be considered as hops of average composition ranging from 15.60 to 18.14 per cent of total soft resins. The use of 7 ml. of lead acetate reagent in the determina-

tion of the alpha resin content of these samples produced results that compare closely with those obtained by the spot test method, with deviations of +1.8, +0.9, and -3.5 per cent, respectively, from the actual content of alpha resins contained in them. Sample 1, chosen because of its exceptionally high content of total soft resins (22.28 per cent) with a deficiency of 1 ml. of lead acetate reagent shows a deviation of -6.6 per cent, whereas Samples 5 and 6, which were unusually low in soft resins, containing 14.37 and 13.18 per cent, respectively, show much greater deviations (-31 and -48.4 per cent) from the actual content of alpha resins when 7 ml. of lead acetate reagent, which is an excess of 3 ml., was used for precipitation of their alpha lead salts. Hops with very high or low content of soft resins will require correspondingly more or less of the reagent for satisfactory results, depending upon the actual content of soft resins found in them. Inasmuch as the total soft resin content of any particular sample of hops is always determined prior to the alpha resin content, it should not be difficult for an analyst, from the information given in Table 1, to judge the correct quantity of lead acetate solution to be used for the rapid determination of the alpha resin content by direct addition of the reagent. However, if a hop sample is found to contain a high total soft and an unusually low alpha resin content, which is sometimes the case, it may be necessary to predetermine the exact quantity of lead acetate reagent to be used. For the purpose of saving time, which is an important factor in hop analysis, the more rapid yet comparatively accurate method of direct addition of a fixed quantity of lead acetate reagent, depending upon the percentage of soft resins found in the hops, is recommended.

## REPORT ON SUBSIDIARY DYES IN D&C COLORS

### 1,4 (*o*-SULFO-*p*-TOLUINO)-ANTHRAQUINONE IN D&C GREEN NO. 5

By LOUIS KOCH (H. Kohnstamm & Co., Inc., Brooklyn, N. Y.),  
*Associate Referee*

D&C Green No. 5, 1,4-Bis(*o*-sulfo-*p*-toluino)-anthraquinone, may contain partly sulfonated material; i.e., one or none of the two *p*-toluidine molecules condensed with leuco quinizarin may be sulfonated. Jablonski (*This Journal*, 25, 230) has shown that D&C Green No. 6 can be split and reduced quantitatively to yield an anthraquinone derivative and *p*-toluidine. Similarly, D&C Green No. 5 will give an anthraquinone derivative and *p*-toluidine-*o*-sulfonic acid, any unsulfonated color resulting in the formation of *p*-toluidine as well.

The unsulfonated amine can readily be separated from other reduction products by extraction with ether from an alkaline solution, and can then be estimated bromometrically.

## EXPERIMENTAL

## REAGENTS

*Compound acetic acid.*—Glacial acetic acid 10 volumes, conc. HCl 2 volumes, and water 1 volume.

*Stannous chloride solution.*—100 grams of  $\text{SnCl}_2$  per 100 ml. of conc. HCl solution.

*Sodium hydroxide solution.*—50%.

*Sulfuric acid.*—Approximately 0.3 N.

*Potassium bromate-bromide solution.*—0.05 N. 1.3920 grams of  $\text{KBrO}_3$  and 10 grams of KBr per liter. Standardize against freshly distilled *p*-toluidine as follows: Dissolve 0.50 gram of freshly distilled *p*-toluidine in 100 ml. of ca. 0.3 N  $\text{H}_2\text{SO}_4$ , and dilute to 1 liter. Transfer a 10 ml. aliquot to a 500 ml. iodine flask, and dilute to 200 ml. with the  $\text{H}_2\text{SO}_4$  soln. Heat to  $70^\circ\text{C}$ . and run in ca. 20 ml. of the  $\text{KBrO}_3$ -KBr solution as rapidly as possible, from a buret. Continue as directed in the procedure for the estimation of the monosulfonated dye in D&C Green No. 5, beginning with the words "Stopper and let stand 5 minutes." Calculate the value of the  $\text{KBrO}_3$ -KBr solution in terms of mg. of *p*-toluidine per ml.

*Sodium thiosulfate solution.*—Approximately 0.05 N. 12.5 grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  per liter. Standardize against the  $\text{KBrO}_3$ -KBr solution as follows: Heat 200 ml. of the  $\text{H}_2\text{SO}_4$  solution to  $70^\circ\text{C}$ . and run in ca. 20 ml. of the  $\text{KBrO}_3$ -KBr solution as rapidly as possible. Continue as directed in the procedure for the estimation of the monosulfonated dye in D&C Green No. 5, beginning with the words, "Stopper and let stand 5 minutes." Calculate the value of the  $\text{Na}_2\text{S}_2\text{O}_3$  solution in terms of the  $\text{KBrO}_3$ -KBr solution.

*Potassium iodide solution.*—10%.

*Starch indicator.*—0.5% solution.

## PROCEDURE

Weigh 5.0 grams of sample into a 250 ml. narrow-necked Erlenmeyer, and add 40 ml. of the compound acetic acid and 5 ml. of the  $\text{SnCl}_2$  solution. Boil gently until the volume is ca. 15 ml. Dilute the reaction mixture with 100 ml. of water and transfer to a 200 ml. volumetric flask. Cool, and dilute to volume. Filter off a 100 ml. aliquot and place it in a 500 ml. extraction funnel. Make the sample alkaline with 25 ml. of a 50% NaOH solution, cooling the funnel during the addition of the alkali. Extract the liberated amine with two 100 ml. portions of ether.

Combine the ether extracts and wash them with four 25 ml. volumes of water. Remove the amine from the ether with four 50 ml. portions of ca. 0.3 N  $\text{H}_2\text{SO}_4$ , and transfer the acid washings to a 500 ml. iodine flask, previously marked at the 200 ml. point. Boil to expel dissolved ether, and dilute with water, if necessary, to the 200 ml. mark. Cool to  $70^\circ\text{C}$ ., and add ca. 35 ml. of the  $\text{KBrO}_3$ -KBr solution from a buret, as rapidly as possible. Stopper immediately and let stand 5 minutes. Add 10 ml. of the KI solution carefully, to avoid loss of bromine. Insert a thin strip of paper between the stopper and the flask, and cool to room temperature. Back titrate the liberated iodine with the  $\text{Na}_2\text{S}_2\text{O}_3$  solution, adding a few drops of the starch indicator near the end point.

Repeat the analysis, using an aliquot that will consume 20 ml. or less of the  $\text{KBrO}_3$ -KBr solution, adding ca. 5 ml. excess. (Best results are obtained under these conditions.)

$$\frac{\text{mg. of } p\text{-toluidine} \times 4.858 \text{ (factor)}}{\text{wt. of sample in mg.}} \times 100 = \% \text{ monosulfonated dye.}$$

Completely unsulfonated D&C Green No. 5 can be isolated as part of the water-insoluble matter, and can then be estimated in a manner analogous to the one used for the lower sulfonated dyes. Subtracting the mg. of *p*-toluidine found in the water-insoluble part from the total *p*-toluidine in the D&C Green No. 5 will give the true 1,4 (*o*-sulfo-*p*-toluino)-anthraquinone content of the primary color. In most instances this additional procedure may not be necessary, because the maximum water-insoluble matter permitted is 1.0%, and the majority of the certified dyes contain considerably less than the permitted maximum.

$$\frac{\text{mg. of } p\text{-toluidine} \times 1.955 \text{ (factor)}}{\text{wt. of sample in mg.}} \times 100 = \% \text{ unsulfonated dye.}$$

## RESULTS

Varying quantities of *p*-toluidine were added to 200 ml. of ca. 0.3 *N* sulfuric acid solution, and titrated according to the proposed bromometric method. The recoveries are given in Table 1.

TABLE 1

P-TOLUIDINE ADDED	VOL. OF KBrO <sub>3</sub> -KBr ADDED	VOL. OF Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> USED IN BACK TITRATION	P-TOLUIDINE FOUND	ERROR
mg.	ml.	mg.	mg.	mg.
0.25	5.10	4.85	0.21	-0.04
0.50	5.70	5.20	0.42	-0.08
0.75	5.70	4.75	0.81	+0.06
1.00	5.90	4.75	0.98	-0.02
1.25	5.70	4.25	1.23	-0.02
2.50	8.80	5.85	2.50	0.00
5.00	12.30	6.55	4.87	-0.13
10.00	15.90	4.25	9.87	-0.13
12.50	19.15	4.35	12.54	+0.04
15.00	22.65	5.00	14.96	-0.04
20.00	30.25	6.15	20.42	+0.42
25.00	35.80	5.45	25.72	+0.72

Five grams of D&C Green No. 5 were analyzed according to the proposed method, pure *p*-toluidine being added to three samples to test the effective amine recovery. The findings are outlined in Table 2.

## DISCUSSION

The bromination of *p*-toluidine is a standard procedure for its estimation. It absorbs 2 atoms of bromine, at room temperature, forming a di-bromo-*p*-toluidine, the end point of the reaction being detected with starch-potassium iodide paper. Attempts to duplicate the procedure of Callan, Russel, and Henderson<sup>1</sup> did not give satisfactory results, either by spotting on the starch-potassium iodide paper, or by back titrating

<sup>1</sup> *J. Soc. Chem. Ind.*, 41, 161T (1922).

the iodine liberated from excess potassium bromate-bromide solution with sodium thiosulfate, using starch solution as an indicator. In the latter case the return of the blue iodine-starch complex, after complete decolorization with the sodium-thiosulfate solution, was so rapid that an accurate observation was impossible.

Experiments conducted by the Associate Referee indicated that more than the theoretical quantity of bromine required for a di-bromo deriva-

TABLE 2

WT. OF DYE ALIQUOT	<i>p</i> -TOLUIDINE ADDED	VOL. OF KBrO <sub>3</sub> ADDED	VOL. OF Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> USED IN BACK TITRATION	<i>p</i> -TOLUIDINE IN DYE	ADDED <i>p</i> -TOLUIDINE FOUND†	MONO-SULF. DYE
grams	mg.	ml.	ml.	mg.	mg.	per cent
2.50	None	35.10	3.85	26.48	—	5.15
1.25	"	21.25	5.50	13.35	—	5.19
1.25	"	20.40	4.80	13.22	—	5.14
1.25	3.125	25.60	6.25	16.47*	3.13	—
1.25	3.125	25.20	5.95	16.31*	3.04	—
0.625	1.563	13.50	3.90	8.14*	1.51	—

\* *p*-Toluidine in dye plus *p*-toluidine added.

† Subtracted average *p*-toluidine content of dye from the total *p*-toluidine found.

tive was being absorbed, and that the rate of this absorption depended upon (1) time, (2) temperature, and (3) acid concentration. Actual bromine consumption in the proposed method was found to be 6.3 atoms. Extraction of the reaction mixture with ether gave a product that recrystallized from water in rhombs and had a M.P. of 135°–136°C. and a bromine content of 56.4 per cent. Calculated for C<sub>7</sub>H<sub>7</sub>Br<sub>2</sub>No, the bromine content is 56.9 per cent. The dibromo derivative of *p*-toluidine has a M.P. of 73°C.

A review of the literature did not disclose a compound that was identical with the one isolated. Beilstein<sup>2</sup> listed an interesting finding by Widman, which stated that *o*-toluidine containing *p*-toluidine gave a compound C<sub>7</sub>H<sub>6</sub>Br<sub>3</sub>N (6 atoms of bromine consumed) when brominated hot, and that on warming the compound with ethyl alcohol it formed the ethyl ether.

It is recommended that the proposed method for the determination of the monosulfonated dye in D&C Green No. 5 be studied collaboratively.

<sup>2</sup> "Handbuch der Organischen Chemie," 3rd ed., Vol. 2, p. 1063.

## REPORT ON ARSENIC IN HAIR LOTIONS

By HAROLD LEE BURRILL (State Board of Barbers and Hairdressers,  
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*Part I*

The question of arsenic in hair lotions involves its determination in quantities varying from 0.01 to 1.0 per cent, in aqueous solutions and in those containing oils and fats.

Working samples of each type of lotion were prepared in the laboratory by the Associate Referee as follows:

<i>Aqueous type</i>	<i>grams</i>
Salicylic acid	2
Betanaphthol	3
Borax	10
Quinine sulfate	1
Glycerol	3
Industrial spirits	500
Distilled water q.s. to	1000
Tinted with brilliant blue FCF certified	
Let stand a few days and filter clear.	

<i>Oil type</i>	<i>grams</i>
Salicylic acid	2
Industrial spirits	500
Mineral oil	9
Distilled water q.s. to	1000

<i>Soap type</i>	<i>grams</i>
Chloral hydrate	6
Resorcinol	4
Cresol	10
Soft soap base	40
Industrial spirits	120
Distilled water q.s. to	1000

These solutions were used as a base. Known volumes of the base were measured out, and the arsenic was added in the form of standard potassium arsenite solution.

A 10 per cent potassium arsenite solution was prepared and standardized against iodine, which had previously been standardized against 0.1 N sodium thiosulfate.

There was needed 1500 ml. of each dilution strength. The potassium arsenite was pipetted into the volumetric flask and the lotion was added to volume.

<i>per cent</i>	<i>ml. of KAsO<sub>3</sub></i>	<i>ml.</i>
0.01 soln =	1.56 + lotion to 1500	
0.1 soln =	15.62 + lotion to 1500	
0.5 soln =	78.10 + lotion to 1500	
1.0 soln =	156.20 + lotion to 1500	

This gives three bottles of each percentage strength and a fourth bottle for a blank.

The first experiments were made with several variations of the Gutzeit method applied to samples containing 0.01 per cent of arsenic trioxide. In most cases low percentage recoveries of arsenic were obtained, especially where the samples had been previously treated with sulfuric and nitric acid and heated to sulfur trioxide fumes. (The arsenic may be tied up or it may be lost in heating.) This was especially noticeable when samples of a standard arsenic solution were used directly and after treatment with the acids. A report of these experiments follows:

*General conditions for all experiments:*

Wide-mouthed 3 ounce bottles were used as generators.

All samples were prepared in the laboratory.

All dishes, pipets, bottles, etc. were washed with 30% NaOH, rinsed with distilled water, washed with (1+1) HNO<sub>3</sub>, and rinsed with distilled water before use.

All precautions were taken to prevent contamination.

Fresh dilute solutions were prepared at frequent intervals.

A constant temperature bath was used to keep uniform temperature during the evolution of the arsenous hydride and to control the temperature in making up the standard arsenic stains. Two sets of standard stains were made for each method, and from these one set of uniform standard stains was selected.

### EXPERIMENTS

*Experiment No. 1.*—Sample used "as is."

As<sub>2</sub>O<sub>3</sub> added: 0.01% to each sample.

Volumes used: 10 ml. of sample, 50 ml. of water, 5 ml. of H<sub>2</sub>SO<sub>4</sub>, 5 ml. of 15% KI, and 4 drops of SnCl<sub>2</sub> · 2H<sub>2</sub>O.

Zinc used: 15 grams of stick zinc.

Temperature: 21°C.

Time of evolution: 90 min.

TABLE 1.—Results by A.O.A.C. method<sup>1</sup>

SOLUTION USED	NO. OF TRIALS	AV.	As <sub>2</sub> O <sub>3</sub> RECOVERED	
			MAX.	MIN.
		per cent	per cent	per cent
Standard As <sub>2</sub> O <sub>3</sub>	16	99	100	90
Oil-type lotion	16	106	132	99
Water-type lotion	16	98	102	81
Soap-type lotion	11*	122	156	93

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930.

\* On additional 5 trials the stains were not sharp enough to be read.

*Experiment No. 2.*—Sample of 10 ml. was evaporated to dryness in porcelain casserole and cooled; 5 ml. of H<sub>2</sub>SO<sub>4</sub> and 5 ml. of HNO<sub>3</sub> were added and the mixture was evaporated to SO<sub>3</sub> fumes and cooled; 50 ml. of water and 25 ml. of saturated (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> solution was added; the mixture was evaporated to fumes and cooled; and the procedure was continued as in Experiment No. 1.

Temperature: 23°C.

TABLE 2.—*Results by modified method*

SOLUTION USED	NO. OF TRIALS	AV.	As <sub>2</sub> O <sub>3</sub> RECOVERED MAX.	MIN.
		per cent	per cent	per cent
Standard As <sub>2</sub> O <sub>3</sub>	16	66	100	30
Oil-type lotion	16	69	105	39
Water-type lotion	16	98	130	86
Soap-type lotion	16	67	87	51

*Experiment No. 3.*—Same conditions as in Experiment No. 2 except that the second evaporation to SO<sub>2</sub> fumes was omitted and the evolution took place at 21.5°C.

TABLE 3.—*Results by modified method*

SOLUTION USED	NO. OF TRIALS	AV.	As <sub>2</sub> O <sub>3</sub> RECOVERED MAX.	MIN.
		per cent	per cent	per cent
Standard As <sub>2</sub> O <sub>3</sub>	16	71	90	40
Oil-type lotion	16	78	99	49
Water-type lotion	16	99	108	76
Soap-type lotion	16	97	103	83

*Experiment No. 4.*—Conditions same as in Experiment No. 2 except that before the second evaporation to SO<sub>2</sub> fumes only 15 ml. of water was added.

*Temperature:* 21.4°C.

TABLE 4.—*Results by modified method*

SOLUTION USED	NO. OF TRIALS	AV.	As <sub>2</sub> O <sub>3</sub> RECOVERED MAX.	MIN.
		per cent	per cent	per cent
Standard As <sub>2</sub> O <sub>3</sub>	16	72	100	40
Oil-type lotion	16	60	77	39
Soap-type lotion	16	85	98	62

*Experiment No. 5.*—Sample of 10 ml. was evaporated to dryness in porcelain casserole and cooled; 5 ml. of H<sub>2</sub>SO<sub>4</sub> and 5 ml. of HNO<sub>3</sub> were added, and the mixture was evaporated to SO<sub>2</sub> fumes and cooled; 15 ml. of water was added, the mixture was evaporated to fumes and cooled, and the procedure was continued as directed in *Methods of Analysis, A.O.A.C., 1920.*

TABLE 5.—*Results by old A.O.A.C. method*

SOLUTION USED	NO. OF TRIALS	AV.	As <sub>2</sub> O <sub>3</sub> RECOVERED MAX.	MIN.
		per cent	per cent	per cent
Standard As <sub>2</sub> O <sub>3</sub>	16	77	100	50
Oil-type lotion	16	58	89	33
Water-type lotion	16	103	108	92
Soap-type lotion	16	73	92	51

*Experiment No. 6.*—Same as Experiment 5 except that 8 drops of SnCl<sub>2</sub> · 2H<sub>2</sub>O were used.



TABLE 6.—Results by modification of old A.O.A.C. method

SOLUTION USED	NO. OF TRIALS	As <sub>2</sub> O <sub>3</sub> RECOVERED		
		AV.	MAX.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard As <sub>2</sub> O <sub>3</sub>	8	61	95	40
Oil-type lotion	8	63	100	22
Water-type lotion	8	107	108	103
Soap-type lotion	8	54	62	41

*Experiment No. 7.*—Same as Experiment No. 5 except that 0.5 ml. of SnCl<sub>2</sub> · 2H<sub>2</sub>O was used.

TABLE 7.—Results by modification of old A.O.A.C. method

SOLUTION USED	NO. OF TRIALS	As <sub>2</sub> O <sub>3</sub> RECOVERED		
		AV.	U. X.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard As <sub>2</sub> O <sub>3</sub>	8	62	85	30
Oil-type lotion	8	63	100	44
Water-type lotion	8	121	130	97
Soap-type lotion	8	69	92	51

*Experiment No. 8.*—Sample used "as is," As<sub>2</sub>O<sub>3</sub> added: 0.01% in each sample.  
*Volume used:* 10 ml. of sample, 50 ml. of water, 5 ml. of H<sub>2</sub>SO<sub>4</sub>, 2 ml. of ferric ammonium sulfate, and 1 ml. of SnCl<sub>2</sub> · 2H<sub>2</sub>O.

*Zinc used:* 5 grams of mossy zinc.

*Temperature:* 20.5°C.

*Time of evolution:* 30 min.

TABLE 8.—Results by modified Scott method<sup>1</sup>

SOLUTIONS USED	NO. OF TRIALS	As <sub>2</sub> O <sub>3</sub> RECOVERED		
		AV.	MAX.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard As <sub>2</sub> O <sub>3</sub>	16	116	120	100
Oil-type lotion	16	107	121	99
Water-type lotion	16	118	130	87
Soap-type lotion	3*	98	98	98

<sup>1</sup> "Standard Methods of Chemical Analysis," (1917).

\* On additional 13 trials the stains were not sharp enough to be read.

*Experiment No. 9.*—Sample of 10 ml. was evaporated to dryness in porcelain casserole and cooled; 5 ml. of H<sub>2</sub>SO<sub>4</sub> and 5 ml. of HNO<sub>3</sub> were added; and the mixture was evaporated to SO<sub>3</sub> fumes. Procedure was continued as in Experiment No. 8.

*Temperature:* 20.5°C.

*Time of evolution:* 45 minutes. Papers were reversed and evolution was continued 45 minutes longer. The total stains on the paper were reported.

TABLE 9.—Results by modified Scott method

SOLUTION USED	NO. OF TRIALS	As <sub>2</sub> O <sub>3</sub> RECOVERED		
		AV.	MAX.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard As <sub>2</sub> O <sub>3</sub>	16	64	75	50
Oil-type lotion	16	70	104	28

*Experiment No. 10.*—Same as Experiment No. 9, except that before the second evaporation to  $\text{SO}_2$  fumes 50 ml. of water and 25 ml. of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  solution were added. The mixture was evaporated to fumes.

*Temperature:* 21.5°C.

TABLE 10.—*Results by modified Scott method*

SOLUTION USED	NO. OF TRIALS	$\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard $\text{As}_2\text{O}_3$	16	82	110	50
Oil-type lotion	16	63	105	33
Soap-type lotion	16	64	82	51

*Experiment No. 11.*—Same as Experiment No. 9 except that the second evaporation to  $\text{SO}_2$  fumes was omitted and the evolution took place at 40°C.

*Time of evolution:* 30 min.

TABLE 11.—*Results by Scott method*

SOLUTION USED	NO. OF TRIALS	$\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Standard $\text{As}_2\text{O}_3$	9*	77	95	60
Oil-type lotion	16	55	77	33
Water-type lotion	16	96	108	22
Soap-type lotion	16	54	87	31

\* On additional 7 trials the stains were not sharp enough to be read.

## CONCLUSION

In the opinion of the Associate Referee the Gutzeit method is not suitable for the determination of arsenic in hair lotions because of unpredictable experimental results and losses of arsenic, particularly in the presence of organic matter. Further experiments were therefore conducted with the method of Cassil and Wichmann (*This Journal*, 22, 436-445).

## Part II

### METHOD OF CASSIL AND WICHMANN

*Dilutions for arsenic and iodine stock solutions:*

<i>micro-</i> <i>grams/ml.</i>	<i>ml.</i>	
50	10.01	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	5.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.
100	20.02	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	10.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.
200	40.04	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	20.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.
300	60.06	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	30.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.
400	80.08	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	40.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.
500	100.1	$\text{As}_2\text{O}_3$ stock soln. + $\text{H}_2\text{O}$ to 200 ml.
	50.	$\text{I}_2$ stock soln., 6.25 grams of $\text{KI}$ + $\text{H}_2\text{O}$ to 250 ml.

TABLE 12.—*Results of blanks*  
 50 micrograms  $As_2O_3$ /ml.  
 50 ml. sample of blank +  $H_2O$  to 1000 ml. 10 ml. aliquots taken

DISTILLED $H_2O$				AQUEOUS TYPE				SOAP TYPE				OIL TYPE			
NO. OF TRIALS	MICROGRAMS OF $As_2O_3$		NO. OF TRIALS	MICROGRAMS OF $As_2O_3$		NO. OF TRIALS	NO. OF TRIALS	MICROGRAMS OF $As_2O_3$		NO. OF TRIALS	NO. OF TRIALS	MICROGRAMS OF $As_2O_3$		AY.	MAX. MIN.
	AV.	MAX. MIN.		AV.	MAX. MIN.			AV.	MAX. MIN.			AV.	MAX. MIN.		
5	1.74	2.59 1.30	5	2.21	2.34 2.11	5	5	2.53	2.61 2.24	5	5	1.96	2.00 1.89		
100 mg. $As_2O_3$ /ml.															
5	2.22	2.89 1.69	5	3.95	4.57 3.37	5	5	1.93	1.93 1.93	5	5	2.65	2.90 2.17		
200 mg. $As_2O_3$ /ml.															
5	3.16	3.33 2.90	5	2.12	2.90 1.44	5	5	2.31	3.33 1.93	5	5	3.24	3.33 2.90		
300 mg. $As_2O_3$ /ml.															
5	2.46	2.90 2.17	5	1.74	2.90 1.45	5	5	3.91	5.06 2.17	5	5	8.68	8.68 8.68		
400 mg. $As_2O_3$ /ml.															
5	6.04	7.80 4.87	5	1.55	1.94 0.97	5	5	5.04	6.78 2.91	5	5	5.26	5.84 4.87		
500 mg. $As_2O_3$ /ml.															
5	4.13	4.86 3.65	5	9.25	10.96 7.31	5	5	5.42	8.62 3.70	5	5	10.61	12.34 8.62		

After blanks had been run on distilled water and the three types of lotions, a series of tests was made on known samples of arsenic in distilled water, the iodine being added and the back titrations being made with known arsenic solutions.

Weak arsenic solutions were prepared to contain from 50 to 500 micrograms/ml.; 1 ml. of this equivalent arsenic solution was run into a generator flask from a microburet, water and other reagents were added, heat was applied, and arsine was collected in the absorbing solution. The same running conditions were maintained as for blanks and samples.

To get the true recovery in micrograms, from these titrations deductions were made for the blanks obtained by running distilled water.

The results are given in the tables.

*Experiment No. 12.*—Sample used "as is."

*Volumes used:* 10 ml. of HCl, 5 ml. of KI, 1 ml. of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , and 60 ml. of  $\text{H}_2\text{O}$ , plus aliquot of the blank.

*Zinc used:* 4 grams of 30-mesh zinc.

*Temperature:* Boiling.

*Time of evolution:* 7 min.

*Experiment No. 13.*—Same as Experiment No. 12 except that trials were made on samples containing from 50 to 500 micrograms. In the 500 microgram trials the zinc was increased to 5. grams, and the absorbing solution was increased to 2 ml.

TABLE 13.—Results of standard  $\text{As}_2\text{O}_3$  solutions

$\text{As}_2\text{O}_3$ ADDED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
<i>micrograms</i>							
50	12	48	49	47	96	98	95
100	12	95	97	94	95	97	94
200	12	194	196	192	97	98	96
300	12	294	297	291	98	99	97
400	12	387	390	386	97	98	97
500	12	495	498	493	99	100	99

*Experiment No. 14.*—Sample used "as is."

*$\text{As}_2\text{O}_3$  added:* 100 micrograms to each sample.

*Volumes used:* 10 ml. HCl, 5 ml. KI, 1 ml.  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , 60 ml.  $\text{H}_2\text{O}$ , 5 ml. aliquot of sample.

*Zinc used:* 4. grams 30-mesh zinc.

*Temperature:* Boiling.

*Time of evolution:* 7 min.

TABLE 14

SOLUTIONS USED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	95	96	95	95	96	94
Soap-type lotion	8	95	97	94	95	97	94
Oil-type lotion	8	95	99	92	95	99	92

*Experiment No. 15.*—Sample of 5 ml. aliquot (100 mmg.) was evaporated to dryness in a porcelain casserole and cooled; 5 ml. of  $\text{H}_2\text{SO}_4$  and 5 ml. of  $\text{HNO}_3$  were added; and the mixture was evaporated to  $\text{SO}_3$  fumes. Procedure was continued as in Experiment No. 14.

TABLE 15

SOLUTIONS USED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	82	91	74	82	91	74
Soap-type lotion	16	70	100	32	70	100	32
Oil-type lotion	8	86	97	61	86	97	61

*Experiment No. 16.*—Same as Experiment No. 14 except that 200 micrograms of  $\text{As}_2\text{O}_3$  was added to each sample.

TABLE 16

SOLUTIONS USED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	193	198	187	97	99	93
Soap-type lotion	8	199	201	198	100	101	99
Oil-type lotion	8	194	195	193	97	98	96

*Experiment No. 17.*—Same as Experiment No. 15 except that 200 micrograms of  $\text{As}_2\text{O}_3$  was added to each sample.

TABLE 17

SOLUTIONS USED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	172	180	161	86	90	83
Soap-type lotion	8	78	114	53	39	57	27
Oil-type lotion	8	184	192	175	92	96	87

*Experiment No. 18.*—Same as Experiment No. 14 except that 400 micrograms of  $\text{As}_2\text{O}_3$  was added to each sample.

TABLE 18

SOLUTION USED	NO. OF TRIALS	MICROGRAMS $\text{As}_2\text{O}_3$ RECOVERED			% $\text{As}_2\text{O}_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	395	403	391	99	101	97
Soap-type lotion	16	393	396	391	98	99	97
Oil-type lotion	8	398	401	395	99	100	99

*Experiment No. 19.*—Same as Experiment No. 15 except that 400 micrograms of  $As_2O_3$  was added to each sample.

TABLE 19

SOLUTION USED	NO. OF TRIALS	MICROGRAMS $As_2O_3$ RECOVERED			% $As_2O_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	366	368	362	91	92	90
Soap-type lotion	16	270	325	229	68	81	57
Oil-type lotion	16	386	400	366	97	100	91

*Experiment No. 20.*—Same as Experiment No. 14 except that 500 micrograms of  $As_2O_3$  was added to each sample; 5 grams of zinc and 2 ml. of absorbing solution were used.

TABLE 20

SOLUTION USED	NO. OF TRIALS	MICROGRAMS $As_2O_3$ RECOVERED			% $As_2O_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	494	499	492	99	100	98
Soap-type lotion	8	490	494	489	98	99	98
Oil-type lotion	8	501	505	492	100	101	98

*Experiment No. 21.*—Same as Experiment No. 15 except that 500 micrograms of  $As_2O_3$  was added to each sample; 5 grams of zinc and 2 ml. of absorbing solution were used.

TABLE 21

SOLUTION USED	NO. OF TRIALS	MICROGRAMS $As_2O_3$ RECOVERED			% $As_2O_3$ RECOVERED		
		AV.	MAX.	MIN.	AV.	MAX.	MIN.
Aqueous-type lotion	8	490	503	475	98	101	95
Soap-type lotion	16	451	478	427	90	96	85
Oil-type lotion	8	495	497	488	99	99	98

## CONCLUSIONS

Results show that the rapid volumetric micro method used for determining small quantities of arsenic is accurate for hair lotions of the soap, oil, and aqueous types, especially where the samples are taken directly. When samples are evaporated and burned a loss is shown, especially noticeable in the soap-type lotion.

By this rapid method the running time for each sample is materially shortened—less than 10 minutes to complete a sample after its preparation.

When the running time was increased in some instances, the stannous chloride increased, and the amount of zinc increased; no effect was noted

on the results, except in samples containing 500 micrograms, where better recoveries were obtained by increasing the zinc to 5 grams and the absorbing solution to 2 ml.

The apparatus showed a blank with distilled water. This blank must be deducted from the sample to get a true result.

Dilute solutions of arsenic and iodine must be made frequently. Stock solutions must be rechecked occasionally for strength.

This method might be applicable to other types of samples.

The Associate Referee recommends further study of this method and also recommends that other types of samples be used when conditions permit.

The method used was that of C. C. Cassil (Bureau of Entomology and Plant Quarantine) and H. J. Wichmann (Food and Drug Administration), which was given in detail in *This Journal*, 22, 436-445.

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#### CORRECTIONS

In Vol. 26, No. 1, p. 33, 1st column of Table 3 (1st section), change "56" to "51."  
In line 3 of the 3rd paragraph on p. 53, Report on Fertilizers, change "hydrochloric" to "hydrofluoric."

## CONTRIBUTED PAPERS

### DETECTION OF OLIVE OIL IN EDIBLE OIL MIXTURES

By J. FITELSON (U. S. Food and Drug Administration, New York, N. Y.)

The customary method for the detection of olive oil in mixtures of oils depends on the consideration of the usual constants of these mixtures, together with special tests for some of the other edible oils. A direct method for the estimation of olive oil would be more satisfactory, and a review of the tests proposed for the characterization of this oil indicated that further examination of the unsaponifiable constituents, particularly squalene, might provide a basis for such a method.

Bolton and Williams (1) classified oils into four groups, according to the degree of unsaturation of the unsaponifiable matter. Most of the vegetable oils were placed in Group 3 (iodine values 117 to 124), while olive oil, with the highest iodine values (197 to 206), was the sole member of Group 4. However, the examination of a larger number of olive oils by Loew (2), Ricca and Lamonica (3), and Jamieson and McKinney (4) showed an extremely wide range in the iodine values of the unsaponifiable matter, and the usefulness of this method was impaired by the consequent merging of Groups 3 and 4. Later, Thorbjarnerson and Drummond (5) found from 31 to 64 per cent of squalene in the unsaponifiable matter of olive oil, thereby explaining the peculiar Bolton-Williams grouping of this oil. Squalene, an aliphatic hydrocarbon ( $C_{30}H_{50}$ ), contains 6 isolated double bonds and has a theoretical iodine value of 371.

Grossfeld and Timm (6) modified the Bolton-Williams method by determining the iodine absorption of a fraction of the unsaponifiable matter. This fraction, obtained by a single petroleum benzin extraction of the saponified oil under prescribed conditions, contains all the hydrocarbons together with part of the sterols and other unsaponifiable constituents. The presence of considerable material other than squalene in these fractions is shown by the calculated iodine values of 210 to 215 for olive oil and 42 to 88 for other vegetable oils. In an effort to extend the limited data of these investigators, the writer examined several oils by their method but was unable to obtain satisfactory duplicate results. Recently, Kuhn and Gerhard also reported that this method did not give reproducible results (14). These erratic results were probably due to variations in the composition of the unsaponifiable fractions, arising from slight differences in extraction technic. A similar objection to the Bolton-Williams method, where the unsaponifiable matter is obtained by three extractions with petroleum benzin, has been made by Jamieson and McKinney (4).

None of the attempts to devise methods for the quantitative separation of squalene from the other unsaponifiable constituents has been successful. The isolation of characteristic crystals of squalene hexahydrochloride by



direct treatment of olive oil with hydrochloric acid gas was reported by Thorbjarnerson and Drummond (5), but the low recovery and the simultaneous formation of interfering colored material precluded use of this procedure as a satisfactory test. Their successful separation and identification of squalene in olive oil was effected with the aid of a selective adsorption treatment of the unsaponifiable matter, the fraction unadsorbed by the activated alumina consisting almost entirely of this hydrocarbon. Various methods for the separation of squalene from other unsaponifiable constituents of oils were also studied by Täufel, Thaler, and Widmann (8). Although no satisfactory quantitative method was found, a considerable concentration of the squalene was produced by the adsorption treatment.

These previous studies on the selective adsorption method involved the use of relatively large quantities of unsaponifiable matter, unsuitable for routine analytical methods. In the present investigation, it was found that constant fractions could be obtained by the chromatographic treatment of small quantities of unsaponifiable matter and that olive oil could be differentiated from the other common edible vegetable oils by the halogen absorption of the unadsorbed residues. This halogen absorption is assumed to be due to squalene and, as suggested by Grossfeld and Timm (6), the "squalene" content is calculated on the basis of the original oil rather than the unsaponifiable matter. This method of calculation permits shortening of the analytical procedure by the elimination of several weighings and also avoids introduction of another variable showing little correlation with the "squalene" content.

#### EXPERIMENTAL

*Selective Adsorption Treatment.*—In the course of studying the conditions for the chromatographic treatment of the unsaponifiable matter, several types of activated alumina were examined, and all were found to be equally efficient in the concentration of the squalene and removal of sterols and coloring matter. Adsorption columns made with Fisher's adsorption alumina for chromatographic analysis, 80–200 mesh, were easiest to prepare and, since various batches exhibited uniform behavior, this reagent was used in most of the experiments reported here.

Petroleum benzin (b.p. 60°–70°C.) was used to wash the unsaponifiable matter in the adsorption column. The squalene content of the unadsorbed filtrate is affected appreciably by the rate with which the wash solvent passes through the alumina. A rapid washing results in incomplete and variable removal of the squalene. Concordant results, based on the halogen absorption of the filtrates, were obtained when the petroleum benzin was slowly passed through a 10×0.8 cm. column at a rate of about 1 ml. per minute. Since it is known that saturated hydrocarbons and unsaturated hydrocarbons of the squalene type pass most readily through the

column and that prolonged washing may result in bringing unsaturated alcohols into the filtrate (7), experiments were made to establish the minimum volume of solvent necessary for the removal of the squalene from the unsaponifiable matter in 5 grams of oil. Table 1 indicates that 50 ml. of solvent washes essentially all of the hydrocarbon through the column.

TABLE 1.—*Volume of petroleum benzin necessary for selective adsorption separation of squalene from unsaponifiable matter in 5 grams of oil*

TOTAL VOLUME OF SOLVENT USED FOR WASHING	HALOGEN CONSUMPTION OF UNADSORBED RESIDUES (ml. .05 N IODINE)							
	OLIVE OIL		COTTONSEED OIL		PEANUT OIL		CORN OIL	
	(a)	(b)*	(a)	(b)*	(a)	(b)*	(a)	(b)*
ml.								
25	3.84	—	—	—	1.37	—	—	—
50	3.96	3.90	0.32	0.37	1.47	1.39	0.53	0.55
75	4.03	—	—	—	1.51	—	—	—
100	—	3.93	—	—	—	—	0.54	—
175	4.13	—	0.35	—	1.59	—	—	—
500	—	4.11	—	—	—	—	—	—

\* Different batch of alumina used.

*Extraction of the Unsaponifiable Matter.*—In the initial stages of this work, the unsaponifiable matter from 5 grams of oil, extracted by the S. P. A. method (9), was used for the selective adsorption treatment. A more rapid procedure for the removal of the hydrocarbons and part of the other unsaponifiable constituents by a single petroleum benzin extraction of the saponified oil was used by Grossfeld and Timm (6). The content of squalene found in oils when a modification of this shorter procedure with two extractions was used compares favorably with the results obtained by the S. P. A. method. No appreciable differences in results were obtained when four extractions were made by this modified method. The last traces of solvent must be removed from the unsaponifiable matter and the unadsorbed residues in an inert atmosphere in order to avoid air oxidation of the unsaturated constituents.

TABLE 2.—*Comparison of methods for extraction of squalene from 5 grams of oil (mg. squalene found/100 grams of oil)*

EXTRACTION METHOD	OIL					
	OLIVE	OLIVE	PEANUT	COTTONSEED	TEA SEED	CORN
S. P. A.	133	519	24	12	16	19
Petroleum benzin (2 extractions)	135	517	26	13	16	19

*Unsaturation of the Unadsorbed Residue.*—None of the many halogenating agents used to determine the degree of unsaturation is satisfactory for use with all types of unsaturated compounds. Erratic results, influenced largely by the excess of reagents, are obtained on sterols (10) and hydrocarbons (11) by the Hanus and similar methods. The Rosenmund-Kuhnenn method (12), used to a considerable extent in biological work, gives correct values with many sterols (10) and has been adapted for use with small quantities of unsaponifiable matter by Bolton and Williams (1). However, appreciably low but reproducible results have been obtained with this reagent on saponifiable oils with iodine values above 100 (13).

The Hanus and Rosenmund-Kuhnenn methods were applied to several unadsorbed residues and slightly higher values were obtained by the former method. However, in view of the better reproducibility of the results given by the Rosenmund-Kuhnenn method, this rapid and simple procedure has been used to obtain the data reported here. Duplicate determinations have agreed within 7 mg. of squalene per 100 grams of olive oil and within 3 mg. of squalene for other oils. These figures correspond to 0.2 ml. and 0.1 ml. of .05 *N* thiosulfate, respectively, when the halogen absorptions of the unadsorbed residues from 5 grams of oil are determined.

The calculated squalene content of some of the oils was surprisingly high, and further work was done on the nature of the unsaturated constituents in the unadsorbed residues. From 25 to 60 per cent of the unsaponifiable matter from 9 samples of olive oil appeared in the unadsorbed filtrates and consisted almost entirely of squalene. This was shown by iodine values of 336 to 365 and confirmed by the ready formation of the characteristic squalene hexahydrochloride crystals. Much smaller quantities of unadsorbed residue with iodine values of 35 to 209 were obtained from the other vegetable oils and consisted largely of material other than squalene. However, the presence of squalene in those residues was demonstrated by production of the hexahydrochloride crystals and the yields of these crystals indicated that the greater part of the unsaturation was due to squalene.

#### METHOD

##### REAGENTS

(a) *Concentrated potassium hydroxide solution.*—Dissolve 60 grams of KOH in 40 ml. of water.

(b) *Dilute potassium hydroxide solution.*—Dissolve 28 grams of KOH in water and dilute to 1 liter.

(c) *Petroleum benzin.*—Skellysolve B (b.p. 63°–70°C.) or equivalent.

(d) *Aluminum oxide adsorbent, 80–200 mesh.*—Adsorption alumina for chromatographic analysis, Fisher Scientific Co., Pittsburgh, Pa., or equivalent. Keep in tightly closed container, away from moisture.

(e) *Pyridine sulfate bromide reagent.*—0.1 *N*. Dissolve 8 grams of Br in 20 ml. of glacial acetic acid (99.5%). Prepare another solution by adding gradually, with cooling, 5.45 ml. of H<sub>2</sub>SO<sub>4</sub> to a mixture of 20 ml. of glacial acetic acid and 8.15 ml. of pyridine. Mix the two solutions, cool, and dilute to 1 liter with glacial acetic acid.

(f) *Sodium thiosulfate solution*.—0.05 *N*. Dissolve 13 grams of  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in  $\text{CO}_2$ -free water containing 1% of amyl alcohol. Standardize against an exactly 0.05 *N* solution of  $\text{KIO}_3$  (1.7835 grams/liter) as follows: To a glass-stoppered 125 ml. Erlenmeyer flask, add 10 ml. of 10%  $\text{KI}$  solution, 5 ml. of water, 2 grams of  $\text{NaHCO}_3$ , and slowly 5 ml. of ca. 6 *N*  $\text{HCl}$ . Mix, add 25 ml. of the  $\text{KIO}_3$  solution, wash down the sides of the flask with water, and titrate at once with the thiosulfate solution, using starch indicator toward the end of the titration.

(g) *Potassium iodide solution*.—10%.

(h) *Starch indicator*.—1% solution of soluble starch.

#### APPARATUS

*Adsorption Column*.—Prepare immediately before use. Place a small wad of cotton in the constricted end of a glass tube, 0.8 cm. inside diameter and 30 cm. long. Add the adsorption alumina in ca. 10 small portions until a column ca. 10 cm. high is obtained. Apply gentle suction and tamp each portion of the alumina lightly with the flattened end of a heavy glass rod. Place a small wad of cotton on top of the column and tamp lightly. Wash the column with ca. 15 ml. of petroleum benzin, remove the suction, and keep the top of the column covered with a shallow layer of petroleum benzin until ready for use.

#### DETERMINATION

Weigh accurately ( $\pm 20$  mg.) ca. 5 grams of sample into a 125 ml. Erlenmeyer flask, add 3 ml. of the concentrated  $\text{KOH}$  solution and 20 ml. of 95% ethyl alcohol, and boil the mixture under an air condenser for 30 minutes. Cool somewhat, and while still warm, add 50 ml. of petroleum benzin; mix, and transfer to a separator. Rinse the flask with 20 ml. of 95% ethyl alcohol and then with 40 ml. of water, adding the rinsings to the solution in the separator. Shake vigorously, allow the two layers to separate completely, and slowly draw off the soap solution. Pour the petroleum benzin extract from the top of the separator into another separator containing 20 ml. of water. Repeat the extraction of the soap solution with 50 ml. of petroleum benzin. Rotate the combined extracts gently with the 20 ml. of water and, after allowing the layers to separate, discard the wash water. Repeat the washing by shaking vigorously with 20 ml. of water and again discard the lower layer after separation. Wash the petroleum benzin solution with 20 ml. of the dilute  $\text{KOH}$  solution and then with successive 20 ml. portions of water until the wash liquid is free from alkali, shaking vigorously on each occasion. After the final washing, draw off the last drops of water brought down by swirling the separator. Pour the petroleum benzin solution from the top of the separator into a lipped conical beaker. Rinse the separator with petroleum benzin and add rinsings to the beaker contents. Add a few pieces of broken porcelain and evaporate almost all of the solvent on a steam bath. Remove the last traces of solvent in a current of  $\text{CO}_2$ , while warming the beaker.

Dissolve the unsaponifiable matter in 5 ml. of petroleum benzin and transfer to the adsorption tube prepared as described above. (The filtrate, which is caught in a 250 ml. glass-stoppered iodine absorption flask, should emerge dropwise, at a rate of ca. 1 ml. per minute, gentle suction being used if necessary.) When the solution has been nearly drawn into the column, add ca. 5 ml. of the petroleum benzin that has been used to rise the beaker. Continue the addition of the solvent in 5–10 ml. portions, always keeping the surface of the column covered with the liquid, until a total volume of 50 ml. has passed through the adsorption tube. Evaporate most of the solvent in the flask, after adding a few pieces of broken porcelain, and remove the last traces of solvent in an atmosphere of  $\text{CO}_2$ .

Dissolve the unadsorbed residue in 5 ml. of  $\text{CHCl}_3$  and add a quantity of pyridine sulfate bromide reagent sufficient to provide at least 50 % excess (10 ml. will usually be adequate). Allow the mixture to remain in the dark for 5 minutes and then add 5 ml. of 10 % KI solution, together with 40 ml. of water. Mix thoroughly, wash down any free I on the stopper, and titrate with the 0.05  $N$   $\text{Na}_2\text{S}_2\text{O}_3$ . Toward the end of the titration add the starch indicator, shake the flask vigorously, and continue the titration to disappearance of the blue color. Conduct a blank determination on the pyridine sulfate bromide reagent in the same manner and calculate the ml. of 0.05  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  equivalent to the absorbed halogen. A blank determination on all of the reagents used should show practically no consumption of halogen. One ml. of 0.05  $N$   $\text{Na}_2\text{S}_2\text{O}_3$  is equivalent to 1.71 mg. of squalene. Report results as mg. of squalene/100 grams of sample.

### RESULTS AND DISCUSSION

With the exception of 7 authentic samples of olive oil, all of the oils reported here were obtained from commercial shipments and showed normal values by the usual analytical determinations. Many of the olive oil samples were obtained at the time of importation into this country, and some of these samples had been kept in the refrigerator for several years. Particular attention was given to these older samples to prevent inclusion of rancid oils in this work, since such oils show a marked lowering of the squalene content. The range of squalene values found in these older oils was similar to that found in fresh olive oils. Table 3 shows the squalene content of olive oils, arranged according to country of origin.

TABLE 3.—*Squalene content of olive oils*

COUNTRY OF ORIGIN OF OLIVE OILS	SPAIN	ITALY	FRANCE	TU- NISIA	GREECE	MO- ROCCO	PALES- TINE	TUR- KEY	U.S.A. (CALIF.)	UN- KNOWN
No. of oils examined	2	4	2	10	7	2	2	4	5	6
Squalene (mg./100 grams oil) {										
Max.	404	518	388	258	431	370	708	278	448	435
Min.	402	225	381	136	316	352	475	230	159	305
Av.	403	378	385	193	376	361	592	261	335	376

The squalene values for the common edible, refined vegetable oils, together with those for olive oil, are summarized in Table 4. The less

TABLE 4.—*Squalene content of edible vegetable oils*

OIL	OLIVE	COTTON- SEED	PEA- NUT	CORN	SOYA BEAN	SUN- FLOWER	TEA SEED	SES- AME	RAPE
No. of oils examined	44	12	11	9	9	3	3	1	1
Squalene (mg./100 grams oil) {									
Max.	708	12	49	36	17	19	16		
Min.	136	4	13	19	7	8	8		
Av.	330	8	28	28	11	13	12	3	28

commonly used oils, such as sunflower, rape seed, tea seed, and sesame, are included in the tabulation although only a few samples of these oils could be obtained.

The higher squalene content of the olive oils serves to differentiate this oil from the other common edible oils. However, the wide range prevents the use of these data as a diagnostic aid in determining the purity of olive oil. The small unsaturation found in the unadsorbed residues of the edible vegetable oils must also be considered in using the proposed method. However, in conjunction with the usual oil analysis, this method should be of value in confirming the presence of small quantities of olive oil in many types of oil mixtures. Some of the limitations, as well as the applications of this method, are illustrated by the following analysis of 3 mixtures purported to contain olive oil.

<i>Sample</i>	<i>A</i>	<i>B</i>	<i>C</i>
Label claim	80% cottonseed and peanut 20% olive oil	vegetable oil and olive oil	vegetable oil and olive oil
Iodine value (Hanus)	104.7	131.4	126.8
Refractive index (25°)	1.4703	1.4732	1.4729
Cottonseed oil	present	absent	absent
Sesame oil	absent	absent	absent
Tea seed oil	absent	absent	absent
Peanut oil	present (29%)	absent	absent
Squalene (mg./100 grams)	17	11	28

The squalene found in Sample A is the only analytical value that definitely precludes the presence of 20 per cent of olive oil and is the average value expected for a 70 per cent cottonseed-30 per cent peanut oil mixture. Comparison with the lowest values for squalene found in this investigation permits the presence of a maximum of 8 per cent of olive oil. Similarly, the constants found in Sample B are normal for a soya bean oil and the squalene content definitely shows the absence of any appreciable proportion of olive oil. Sample C, with constants similar to those of corn oil, furnishes an example of the limitations of the proposed method. The squalene found here could also be obtained from a mixture of oils with high iodine values, such as soya bean oil, together with small quantities of olive oil. In the absence of specific tests for some of these oils, no definite conclusions concerning the presence of a small quantity of olive oil in this sample can be made.

#### SUMMARY

A simple method of determining the "squalene" content is proposed as an aid in the detection of olive oil in mixtures of the common, edible, vegetable oils. In this method, the squalene is concentrated in a fraction obtained by the selective adsorption treatment of the unsaponifiable matter. The unadsorbed residues from olive oil consist almost entirely

of squalene, and the unsaturated material in the much smaller residues from other oils consists largely of squalene. The unsaturation of these residues is assumed to be due to squalene, and the quantity of this hydrocarbon present in oils is calculated from the total halogen absorption. The results obtained by this method show good reproducibility.

Although all of the vegetable oils examined contained squalene, olive oil is characterized by its much higher squalene content. However, the wide range found in olive oil, together with the small quantities of squalene present in other oils, must be considered in applying the proposed method. In conjunction with the usual chemical analysis, the method should be of value in detecting small quantities of olive oil in many types of oil mixtures.

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#### THE OCCURRENCE OF SQUALENE IN NATURAL FATS

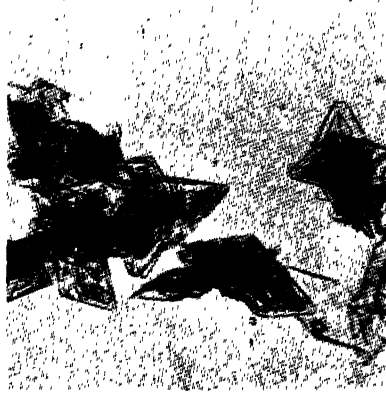
By J. FITELSON (U. S. Food and Drug Administration,  
New York, N. Y.)

In an investigation of methods for the determination of squalene in olive oil (32), it was noted that other vegetable oils contained small quantities of unsaturated material resembling squalene. The presence of squalene in these oils was confirmed by the formation of the characteristic squalene hexahydrochloride crystals from a fraction of the unsaponifiable matter. Since squalene has been reported to be limited to a relatively few fats, mainly those from certain marine animals, the unexpected presence of this compound in these vegetable oils led to the examination of a variety of natural fats.

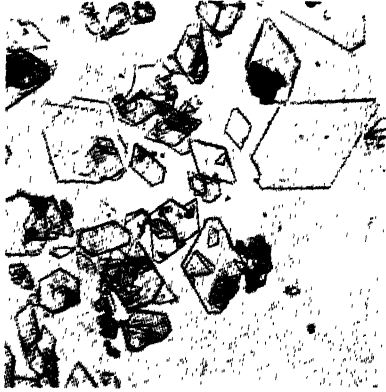
Squalene was found in shark-liver oil in 1906 by Tsujimoto (1). It was



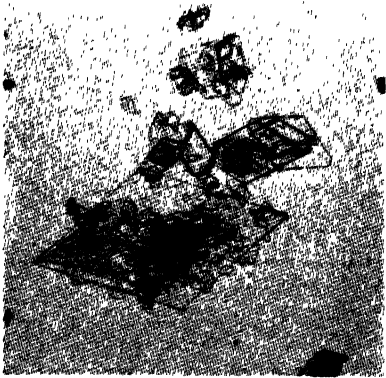




Squalene hexahydrochloride  
from butter fat (125 X)



Squalene hexahydrochloride  
from seal oil (125 X)



Squalene hexahydrochloride  
from olive oil (125 X)

later found in large quantities in the liver oils of 16 out of 36 species of the Elasmobranch fish, chiefly in members of the family *Squalidae* (3). Considerable quantities have also been detected in yeast fat (19). The first references to squalene in vegetable oils appeared in 1935, when small quantities were reported in olive oil (22) and in wheat germ oil (23). Since then, the presence of squalene has been established in only a few of the many fats examined. The literature references on the occurrence of squalene in fats are summarized in Table 1.

TABLE 1.—*Literature references on occurrence of squalene in natural fats*

FATS CONTAINING SQUALENE	FATS NOT CONTAINING SQUALENE
Elasmobranch fish	Elasmobranch fish
Shark liver (various families), (1, 2, 3, 4, 5, 6, 15, 26, 29)	Shark liver (various families), (3, 26, 28, 30, 31)
Rat-fish liver, (15)	Rat-fish liver, (28)
Shark flesh, (7, 8)	Teleostid fish
Shark egg, (6, 8, 9)	Liver from 14 species, (26)
Teleostid fish	Cod liver, (3, 26)
Cod liver, (10, 14, 27, 33)	Herring, (15)
Norway haddock liver, (24)	Phyto- and zoo-plankton, (26)
Bonito, (12)	Mammalian liver, (35)
Ruvettus pretiosus ("castor-oil fish"), (13)	Horse liver, (14, 16)
Whale, (11, 14)	Human serum, (36)
Dermoid cyst, (18, 34)	Human liver, (16)
Yeast, (14, 19, 20)	Human depot, (36)
Wheat germ, (14, 20, 23)	Pig liver, (16, 17)
Olive, (14, 20, 22)	Sheep liver, (16)
Soya, (14,)	Ox liver, (16)
Linseed, (14)	Duck stomach, (21)
Peanut, (14)	Mold, (21)
	Flour worm, (21)
	Ergot, (21)
	Pine nut, (21)
	Hazel nut, (21)
	Cocoa, (21)
	Mandarin seed, (21)
	Wheat germ, (21)
	Rape seed, (14, 20)
	Cottonseed, (14, 20)
	Apple seed, (14)
	Barley germ, (14)
	Rye germ, (14)
	Poppy seed, (14)
	Pumpkin seed, (14)
	Sunflower seed, (14)
	Corn, (14)
	Grape seed, (14)
	Oiticica, (14)
	Soya, (20)

In most of the previous investigations, vacuum distillation and fractional crystallization were used to separate the squalene from the other unsaponifiable constituents. These methods require relatively large quantities of unsaponifiable matter and are not satisfactory for the isolation of very small quantities of squalene. The value of the selective adsorption treatment in the detection of squalene in vegetable fats was demonstrated in 1935 (22, 23). Later, Täufel and Heimann (14) used this method in conjunction with Grossfeld's rapid procedure for the extraction of unsaponifiable matter (25), and succeeded in detecting this hydrocarbon in several other fats. Although no details of their method are reported, it cannot be considered as entirely satisfactory since squalene has now been found in six fats that had been examined by these investigators with negative results.

#### EXPERIMENTAL

In the method used here, the hydrocarbons, together with part of the other unsaponifiable constituents, are extracted from the saponified oil with petroleum benzin by the Grossfeld method (25) and then washed through a column of adsorbent alumina. This treatment results in a concentration of the squalene in the unadsorbed residue. Squalene hexahydrochloride is then formed by passing anhydrous hydrochloric acid through an ether solution of this residue, and it can occasionally be identified microscopically after direct recrystallization from acetone. However, interfering material, probably hydrocarbons, is frequently present at this stage and prevents satisfactory recrystallization of the hydrochloride. These contaminants can be dissolved in low-boiling petroleum benzin, in which squalene chloride is only slightly soluble. Subsequent recrystallization from acetone usually produces the typical diamond or hexagonal shaped plates of the hexahydrochloride. The volumes of solvent used in the recrystallization are governed by the yield of crystals, which can be anticipated by determining the "squalene" content of the fat by the method used for the detection of olive oil (32). The yields are directly proportioned to this "squalene" content.

By the method described here, as little as 2 mg. of squalene in 100 grams of fat has been detected. When sufficient crystals are produced, identification can be made by the melting point as well as microscopically. The melting points of the crystals from many of the fats examined here varied from 110° to 135°C. It is known that squalene hexahydrochloride can occur in several isomeric forms, and melting points from 100° to 150°C. have been reported (5, 6, 22). The crystals obtained from fats examined were compared microscopically with the squalene hexahydrochloride obtained from yeast fat and olive oil.

#### METHOD

Saponify 100 grams of fat by refluxing under an air condenser for 2 hours with 55 ml. of conc. KOH (3+2) and 240 ml. of 95% ethyl alcohol. Cool, and add 300 ml.

of petroleum benzin (b.p. 60°–70°C.). Transfer mixture to a separator, add 240 ml. of 95% ethyl alcohol and 480 ml. of water, and shake vigorously. Allow the layers to separate and transfer the upper layer to another separator. Repeat the extraction of the saponified fat with 300 ml. of petroleum benzin and combine the extracts. Wash the extract twice with 60 ml. portions of water, using gentle agitation. Repeat the washing with 60 ml. of 0.05 N KOH, followed by vigorous agitation with 60 ml. portions of water until the wash liquid is free from alkali. Evaporate the extract on a steam bath, removing the last traces of solvent with a current of CO<sub>2</sub>.

Place a wad of cotton in the constricted end of a glass tube, 0.8 cm. wide and 35 cm. long. Add aluminum oxide (80–200 mesh, especially prepared for chromatographic analysis) in small portions, applying gentle suction and tamping each portion until a column ca. 25 cm. high is obtained, and place a small wad of cotton on the top of the column. Dissolve the unsaponifiable matter in ca. 5 ml. of petroleum

TABLE 2.—*Squalene content of various fats\**

FAT	NO. SAMPLES	SQUALENE† (MG./100 GRAMS FAT)	FAT	NO. SAMPLES	SQUALENE† (MG./100 GRAMS FAT)
Olive	44	136–708	Grapeseed	1	7
Cottonseed	12	4– 12	Almond	1	21
Corn	9	19– 36	Cocoa*	1	none
Peanut	11	13– 49	Coconut	1	2
Sunflower	3	8– 19	Linseed	1	4
Soya bean	9	7– 17	Butter	1	7
Tea seed	3	8– 16	Cod liver	1	31
Sesame	1	3	Seal	1	35
Rape	1	28	Chicken	1	4
Mustard	1	7	Lard	1	3
Patua	2	2– 5	Beef	1	10
Rice bran	1	332			

\* Squalene hexahydrochloride crystals obtained from all fats with exception of cocoa butter, which was not examined microscopically

† Data obtained by method described in paper published on p 499.

benzin (b.p. 60°–70°C.) and wash through the column with this solvent. (The filtrate should emerge dropwise, at a rate of about 1 ml. per minute, and gentle suction should be used if necessary.) Continue washing the column, keeping the surface covered with liquid, until ca. 75 ml. of filtrate is collected. Evaporate the filtrate on a steam bath, removing the last traces of solvent in an atmosphere of CO<sub>2</sub>.

Dissolve the residue in anhydrous ethyl ether and transfer to a 15 ml. conical centrifuge tube, using ca. 2 ml. of the ether. Place the tube in an ice bath and pass in dry HCl gas for 1 hour. Remove the tube from the ice bath and allow the HCl to bubble through the solution at room temperature until the ether has been removed. Add 1 ml. of low-boiling petroleum benzin (35°–60°C.), heat to boiling, cool, and place the tube in a refrigerator. (The length of cooling will depend on the quantity of squalene hexahydrochloride present, very small amounts requiring 24–48 hours for precipitation. Separation of the squalene chloride from interfering waxy substances, which occasionally also precipitate on cooling, can be effected by using a larger volume of petroleum benzin or by allowing the solution to stand at room temperature for several days instead of cooling.)

Remove the solvent by whirling the tube in a centrifuge, decant, and wash once with cold, low-boiling petroleum benzin. Dissolve the residue in a small volume of hot acetone, cool, and place in a refrigerator until crystals appear. Very small yields of crystals require cooling for 1-2 days, after solution in a few drops of hot acetone. If microscopic examination shows the crystals to be imperfect, recrystallize from acetone. (Additional crystals can be obtained by concentrating and cooling the mother liquor.) Examine under the microscope (100 $\times$ ) for typical diamond or hexagonal shaped plates of squalene hexahydrochloride.

## RESULTS

Table 2 lists the "squalene" content of 23 different fats. These data were calculated from the halogen absorption of the unadsorbed residues obtained by the chromatographic treatment of the unsaponifiable matter (32). It will be noted that cocoa butter is the only fat showing no squalene. With the exception of the cocoa butter, which was not further examined, from one to four samples of all the listed fats were analyzed by the method described above, and in every case typical crystals of squalene hexahydrochloride were isolated.

## SUMMARY

A method for the detection of squalene in fats by the formation and microscopic identification of the characteristic hexahydrochloride crystals is described. Concentration of the squalene is effected by a selective adsorption treatment of the unsaponifiable matter with aluminum oxide, the squalene appearing in the unadsorbed filtrate. After being treated with hydrochloric acid gas, the residue is washed with petroleum benzin in order to dissolve contaminating material, and the insoluble chloride is then recrystallized from acetone and examined microscopically. Fats containing as little as 2 mg. of squalene per 100 grams of fat can be successfully examined by this method.

The squalene content of 23 fats was determined, and the results range from none in cocoa butter to a maximum of 708 mg. per 100 grams in one sample of olive oil. With the exception of cocoa butter, these fats were further examined, and typical squalene hexahydrochloride crystals were obtained from all. The fats thus examined included 16 vegetable, 2 marine animal, and 4 land animal fats. Although most of these fats contain very small quantities of squalene, the currently held conception that the occurrence of this hydrocarbon is limited to a few fats, mainly those from certain marine animals, must now be modified.

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## MOLD COUNT RECORDING DEVICE

By GILBERT A. PITMAN (Food and Drug Administration,  
Federal Security Agency, San Francisco, Calif.)

The official method<sup>1</sup> for determining mold in tomato products specifies examination with a microscope at about 100 diameters magnification of twenty-five 1.382 mm. diameter fields on each of two or more Howard slides, the fields being spotted within a 19 mm. circle. As such examination of many samples per day is extremely tiring to many analysts, any technic that would lessen this fatigue is desirable.

Heretofore, the findings in each of the 25 fields of each slide have been recorded by any system deemed appropriate by the analyst or group in-

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1940, 522.

volved, but invariably these systems have necessitated moving the eyes from the microscope to permit manual notation of the presence or absence of mold filaments. Each shift of the eyes to and from the microscope necessitates their refocusing and reaccommodation, and therefore they quickly become fatigued.

Twenty-five mold fields can be distributed over the surface of the 19 mm. disk in such a manner that about  $\frac{1}{8}$  inch separates their centers. A piece of paper fastened to the mechanical stage and moving with the slide can be marked mechanically as each positive field is encountered, and thereby a legible record can be made to show the number and relative position of the fields containing mold filaments without removing the eyes from the microscope.

A device that provides such a record is illustrated here, and its construction is described below. The lower end of a camera cable release is rigidly fastened in a fixed position with respect to the microscope objective and is operated by the left hand (as it supports the forehead) to puncture a microscope slide label that is held on a cork pad on the mechanical stage. The punch marks, being  $\frac{1}{8}$  inch or more apart, are easily counted and marked with pen and ink before the label is fastened to the analytical report.

#### DESCRIPTION OF MECHANISM

A  $3\frac{1}{2}$  inch length of  $\frac{1}{4}$  inch copper tubing was plugged at one end and drilled and tapped for  $\frac{1}{4}$ -40 threads. A piece of thin sheet brass was soldered to the other end of the tube to facilitate its fastening to the prism box of the microscope by means of the screws in the prism box. The lower end of the tube should be no more than  $\frac{1}{8}$  inch above the cork pad of the hinge unit described here. An eight inch camera cable release with a  $\frac{5}{8}$  inch plunger (camera end of the flexible wire) was fastened inside the copper tube by means of the  $\frac{1}{4}$ -40 threads provided. The tip of the plunger was filed to a sharp point. The upper or manually operated end of the cable release was fastened to the prism box between the ocular tubes by reaming a 1 inch length of  $\frac{1}{4}$  inch tubing to make a snug fit on the ferrule of the cable release and soldering this tube to a strip that could be fastened to the prism box with screws.

A small brass hinge with blades  $1\frac{1}{2}$  inch square when closed was used to hold the micro slide label on a piece of pressed cork  $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$  inch. The cork was cemented to the top of the lower blade in such a position that it was centered below a 20 mm. circle cut from the center of the upper hinge blade. The lower surface of the lower blade was soldered to one side of a small piece of angle brass, the other side of which was clamped between the slide holder and its horizontal support.

Approximate "register" was established between the 19 mm. disk of the Howard slide and the 20 mm. circle in the upper hinge blade before the hinge unit was fastened to the mechanical stage. Accurate register was obtained by slightly bending the copper tube supporting the plunger of the cable release.

This description is applicable to the microscope pictured, and with minor alterations it can be applied to the current models of the major American makes. For example, the method of mounting the hinge unit on the model that moves the whole stage requires a solid supporting bar across the two arms that grasp the slide. Another make of microscope



FIG. 1.—MICROSCOPE SHOWING  
ATTACHMENTS

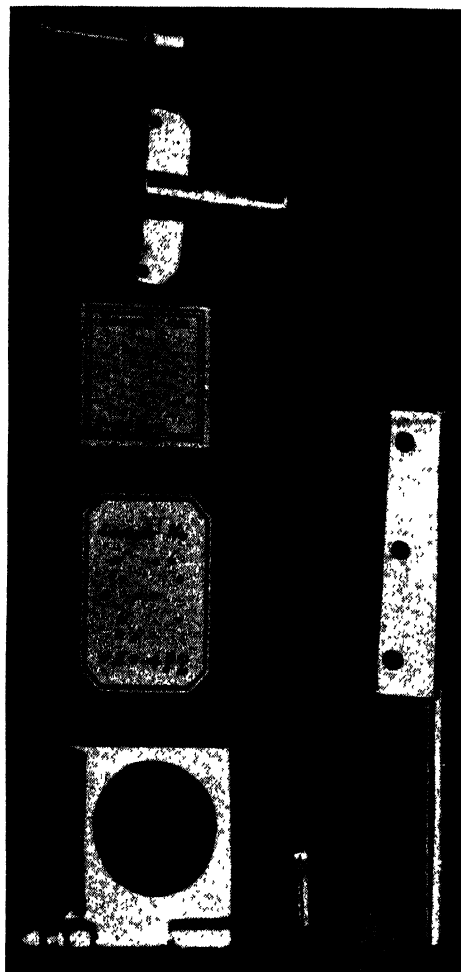


FIG. 2.—ATTACHMENTS  
FOR MICROSCOPE

does not have convenient screws on the prism box, and the long copper tube must be fastened directly to the solid slide behind the prism box by drilling and tapping. Some analysts may prefer to use a long cable release with the manually operated end unattached or fastened at a position more convenient for their posture while counting.

Two incidental advantages accrue from the use of this device. First, there is preserved an accurate record of the geographical distribution of the positive fields on the slide, which may be taken as an indication of a properly (or a poorly) prepared slide; second, when the counting of a slide is interrupted, the field last counted may be determined quickly by glancing at the position of the pointer on the record sticker.



# THE MICROSCOPICAL PROPERTIES OF SOME OF THE CRYSTALLINE WATER-SOLUBLE VITAMINS

By GEORGE L. KEENAN\* (U. S. Food and Drug Administration,  
Washington, D. C.)

The purpose of this paper is to report certain microscopical observations, including optical data and microchemical tests that are of value in the identification of some water-soluble vitamins. The optical constants, including the refractive indices, were determined by the immersion method. The observations usually made with crossed nicols are also recorded. Ascorbic acid, calcium pantothenate, nicotinic acid, nicotinamide, riboflavin, and thiamine hydrochloride were studied. The results obtained are described below.†

## ASCORBIC ACID

*Crystal habit.*—The substance was colorless and the habit was quadratic in outline. For study by the immersion method, the substance was broken up into irregular fragments.

*Refractive indices.*— $n_\alpha = 1.483$ ;  $n_\beta = 1.605$ ;  $n_\gamma = > 1.695$  but  $< 1.734$ ; all  $\pm 0.002$ .

*Characters shown in parallel polarized light with crossed nicols.*—Although many fragments extinguished sharply with crossed nicols, others remained bright under the same conditions.

*Characters shown in convergent polarized light with crossed nicols.*—Partial biaxial interference figures were common, especially those showing one optic axis in the interference figure.

*Distinctive optical characters.*—The frequent occurrence of the minimum and intermediate refractive indices served to identify the substance optically. It should be noted that the immersion method is applicable to powdered tablet material as well as to the pure substance.

*Microchemical test.*—Upon the addition of a drop of gold bromide in HCl reagent‡ to a small quantity of the material on a 3'×1' microscope slide, brownish-black bodies formed. These appeared like very minute black dots in the microscopical field at first but gradually grew larger. In the case of tablet material, they formed more slowly but eventually the field was filled with them.

A small fragment of silver nitrate added to a drop of aqueous solution of ascorbic acid on a microscope slide caused the formation of distinct gray and black plates of hexagonal outline. In the case of tablet material, these hexagons were very small, appearing like small black dots in the preparation. Barnett and Bourne have already called attention to the use of silver nitrate for the histochemical demonstration of ascorbic acid in tissues and cells.§

## CALCIUM PANTOTHENATE

*Crystal habit.*—The substance was colorless and consisted of small rods.

*Refractive indices.*— $n_\alpha = 1.487$ ;  $n_\beta = 1.505$ ;  $n_\gamma = 1.525$ ; all  $\pm 0.002$ .

*Characters shown in parallel polarized light with crossed nicols.*—The extinction was parallel and the sign of elongation was +.

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\* Contribution from Microanalytical Division.

† Samples of vitamins obtained from Vitamin Division, Food and Drug Administration.

‡ To 1 gram of gold chloride add 1.5 ml. of 50% HBr, and then add HCl to make 20 ml. (A saturated solution of NaBr may be substituted for the HBr.)

§ *Nature*, 147, 542-543 (1941).

*Characters shown in convergent polarized light with crossed nicols.*—No interference figures were observed.

*Distinctive optical characters.*—All three indices of refraction were readily found on the substance;  $n_\alpha$  and  $n_\beta$  were shown crosswise on the rods, and  $n_\gamma$  lengthwise.\*

#### NICOTINIC ACID

*Crystal habit.*—The substance was colorless, rod-like, and fibrous in habit.

*Refractive indices.*— $n_\alpha = 1.428 (\pm 0.003)$ ;  $n_\beta = \text{indet.}$ ;  $n_\gamma = > 1.734$ . An intermediate refractive index, which may be designated as  $n_i$ , is 1.734 (methylene iodide).

*Characters shown in parallel polarized light with crossed nicols.*—The extinction was parallel, although it is characteristic of the substance to show many rod-like fragments that do not extinguish sharply with crossed nicols.

*Characters shown in convergent polarized light with crossed nicols.*—Only occasionally were partial biaxial interference figures shown.

*Distinctive optical characters.*—Crystalline nicotinic acid is rather difficult to work with microscopically, and the optical data presented are most useful in a determinative procedure only when combined with the microchemical tests described below. The minimum and intermediate refractive indices, both readily found, are useful in characterizing the pure substance. Some difficulty might be encountered in the case of tablets where the nicotinic acid appears in a finely divided state. In this case the use of a magnification higher than  $\times 200$  may be necessary.

*Microchemical tests.*—The addition of a drop of gold bromide in HCl reagent to a small quantity of pure nicotinic acid on a microscope slide immediately produced an abundance of brown, six-sided prisms, slender rods, and rhombs. The test was very distinctive, and it can be applied also to powdered tablet material.

When a fragment of silver nitrate was added to a small quantity of nicotinic acid in a drop of water on a microscope slide very small burrs, consisting of minute needles, were formed. These are readily observed at  $\times 200$ .

#### NICOTINAMIDE

*Crystal habit.*—The substance was colorless and consisted of small rods.

*Refractive indices.*— $n_\alpha = 1.485 (\pm 0.002)$ ;  $n_\beta = \text{indet.}$ ;  $n_\gamma = > 1.734$ . Like nicotinic acid, an intermediate refractive index,  $n_i = 1.734$  (methylene iodide), was also commonly found, and it proved to be useful in determinative work.

*Characters shown in parallel polarized light with crossed nicols.*—The extinction was parallel and inclined on the rods.

*Characters shown in convergent polarized light with crossed nicols.*—No interference figures were observed.

*Distinctive optical characters.*—Both the minimum and intermediate refractive indices are important constants for identification of nicotinamide microscopically. It should be observed that the minimum refractive index for nicotinic acid ( $n_\alpha = 1.428$ ) is appreciably lower than that for the amide ( $n_\alpha = 1.485$ ).

*Microchemical test.*—Like nicotinic acid, the amide furnished a similar crystalline precipitate when the gold bromide in hydrochloric acid reagent was applied.

#### RIBOFLAVIN

*Crystal habit.*—Riboflavin crystallized in very small, yellow needles.

*Refractive indices.*—It did not lend itself to an accurate determination of the refractive indices. The needles when mounted in a drop of oily immersion liquid developed a vibratory and rotary movement in the menstruum, which precluded accurate determination of the indices.

\* *Lengthwise* = when rod is oriented parallel to vibration plane of the lower nicol. *Crosswise* = when rod is oriented perpendicular to vibration plane of lower nicol.

*Characters shown in parallel polarized light with crossed nicols.*—The needles showed parallel extinction and a characteristic fluorescence when examined with crossed nicols.

*Color reactions.*—When a fragment of solid silver nitrate was placed in a drop of an aqueous solution of riboflavin on a 3'×1' slide, the yellow mixture became gradually a blood-red and this color was permanent. Millon's reagent reacts similarly.

#### THIAMINE HYDROCHLORIDE

*Crystal habit.*—Thiamine hydrochloride was colorless and consisted of plate-like fragments.

*Refractive indices.*—When exposed to the air, anhydrous thiamine hydrochloride rapidly adsorbed moisture and this precluded an accurate determination of the refractive indices by the immersion method.

*Microchemical tests.*—The microscopical identification of thiamine hydrochloride was most conveniently handled by microchemical methods. On addition of a drop of gold bromide in HCl reagent to a small quantity of the material on a microscopical slide, circular aggregates of rods and needles were formed. The test was applicable to tablet material as well as to the pure substance. The characteristic odor of thiamine hydrochloride, even in the tablets, was quite significant and it provided a valuable clue to its presence.

#### SUMMARY

Optical data and microchemical tests on some of the crystalline water-soluble vitamins are presented. These results are of value in the rapid identification of these substances by microscopic examination.

### FURTHER STUDIES OF FACTORS INFLUENCING THE A.O.A.C. CHICK METHOD OF VITAMIN D ASSAY

By I. MOTZOK and D. C. HILL (Department of Animal Nutrition,  
Ontario Agricultural College, Guelph, Canada)

From the results of recent collaborative studies, it has been suggested by Tolle<sup>1</sup> and by Baird and Barthen<sup>2</sup> that further work be done on the A.O.A.C. method of assay for vitamin D to determine how inconsistencies can be lessened. Some of these inconsistencies may be caused by minor variations in the manner in which the bones are treated subsequent to the determination of their ash content. For example, the A.O.A.C. method<sup>3</sup> does not specify in detail how to handle large numbers of bones, such as would be encountered in the economic and practical assay of several oils simultaneously. Under such conditions it may be difficult to follow exactly the same procedure in the preparation of all bones for ashing.

In the course of the vitamin D assay program of this laboratory, such a situation, as well as other problems, was encountered. Moreover, as the writers were not familiar with the unpublished studies that have been con-

<sup>1</sup> *This Journal*, 23, 648-652 (1940).

<sup>2</sup> *Ibid.*, 24, 961-973 (1941).

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1940, 371.

ducted to support the procedure adopted by the A.O.A.C. it was thought advisable to investigate certain of these minor variations in the procedure relative to their influence on the percentage bone ash. The following studies are reported in this paper:

- I. Manner of storage of bones previous to lipid extraction.
- II. Crushing of bones previous to lipid extraction.
- III. Immersion of bones in boiling water.
- IV. Length of ashing period in group-ash technic.

# I. MANNER OF STORAGE OF BONES PREVIOUS TO LIPID EXTRACTION

Lachat<sup>4</sup> expressed the belief that the process of freezing bones does not significantly affect the subsequent estimation of ash. He qualified his observation, however, by stating that any effect from freezing could be shown more conclusively by using a large sample of paired right and left tibiae. Consequently, in the present experiment, 69 pairs of right and left tibiae were used. The left tibiae were immediately cleaned of adhering tissue, extracted, and ashed according to the recommended procedure. The corresponding right tibiae were stored unprepared at a temperature of  $-12^{\circ}\text{C}$ . At the end of 12 days, 36 bones were removed from storage, allowed to thaw out, cleaned, extracted, and ashed; on the 54th day, the remaining 33 bones were treated in a similar manner.

The second method of preservation to be investigated was storage in ethanol as recommended by the A.O.A.C. For this experiment 111 pairs of tibiae were used. The left tibiae were cleaned and stored in 95 per cent ethanol for 24 hours, and then extracted and ashed according to the recommended procedure. The corresponding right tibiae were divided into three groups and stored for 24 hours, 1 week, and 2 weeks, respectively, the first group of right tibiae serving as a check.

The use of paired right and left tibiae for comparative purposes was based on previous findings of Motzok et al.,<sup>5</sup> which showed that there is no significant difference in the percentage ash of paired bones when they are prepared by the regular A.O.A.C. technic.

From the data presented in Table 1 it is evident that the freezing treatment caused a significantly lower ash content. A comparison of the mean weights of the dried bones and of the ash shows that the difference in per cent bone ash can be attributed to a less complete extraction of fat material from the bones that had been frozen. The extension of the storage period to 54 days had little additional effect on the percentage bone ash.

In contrast to the marked effect of cold storage, the preservation of bones in 95 per cent ethanol, at least up to two weeks, had no appreciable effect on their per cent ash. These experiments suggest that when bones

<sup>4</sup> *This Journal*, 20, 450-458 (1937).

<sup>5</sup> *Ibid.*, 25, 965-969 (1942).

TABLE 1.—*Effect of manner of storage on percentage bone ash of chick tibiae*

TREATMENT	PAIRS OF BONES	MEAN WEIGHT OF DRIED BONES	MEAN ASH CONTENT		MEAN DIFFER- ENCE	t* VALUE	PROB- ABILITY†
		grams	grams	per cent	per cent		
None	36	0.3294	0.1305	39.62			
Frozen 12 days		0.3424	0.1309	38.23	1.39	5.74	<0.01
None	33	0.3131	0.1418	45.29			
Frozen 54 days		0.3216	0.1413	43.97	1.32	6.33	<0.01
Stored in ethanol 24 hrs.	36	—	—	38.70			
Stored in ethanol 24 hrs.		—	—	38.23	0.47	1.50	>0.10
Stored in ethanol 24 hrs.	39	—	—	40.83			
Stored in ethanol 7 days		—	—	40.65	0.18	0.52	>0.50
Stored in ethanol 24 hrs.	36	—	—	42.07			
Stored in ethanol 14 days		—	—	42.30	0.23	0.71	>0.10

$$* t = \frac{\bar{d}}{\sqrt{\frac{s^2}{N}}}, \text{ where } \bar{d} \text{ is the mean difference between paired bones, } N \text{ is the number of paired differences,}$$

$$\text{and } s^2 = \frac{S(d^2) - S(d)\bar{d}}{N-1}.$$

† Probability of the significance of the mean difference. A probability of less than 0.05 is usually considered as indicating a significant difference.

cannot be extracted immediately after dissection, storage in alcohol is preferable to freezing as a method of preservation.

## II. CRUSHING OF BONES PREVIOUS TO LIPID EXTRACTION

The official procedure suggests that the crushing of bones may facilitate the extraction of lipid material. When it is necessary to handle a large number of bones, this procedure involves considerable time and labor. To study the degree to which crushing facilitates extraction, 157 pairs of tibiae were used. The right tibiae were crushed before extraction while the left tibiae were extracted whole. The crushing consisted of breaking the bone in half at approximately the mid-point of the shaft and then splitting the two sections longitudinally. Each crushed bone was wrapped separately in filter paper. The extraction procedure consisted of boiling the bones under reflux for 20 hours in 95 per cent ethanol followed by extraction for 20 hours with diethyl ether in a glass Soxhlet apparatus.

TABLE 2.—*Effect of crushing previous to extraction on percentage bone ash of chick tibiae*

TREATMENT	PAIRS OF BONES	MEAN BONE ASH	MEAN DIFFERENCE	<i>t</i> VALUE	PROBABILITY
		<i>per cent</i>	<i>per cent</i>		
Whole tibiae	50	39.60	0.082	1.15	>0.10
Crushed tibiae		39.52			
Whole tibiae	69	39.70	0.014	0.14	>0.5
Crushed tibiae		39.69			
Whole tibiae	88	30.89	0.119	1.18	>0.10
Crushed tibiae		31.00			

From the data in Table 2, it is evident that crushing has no significant effect on the amount of lipid material extracted from bones either at a low or high level of calcification.

### III. IMMERSION OF BONES IN BOILING WATER

To facilitate the removal of adhering tissue from the bones, the official method suggests that the bones may be placed in boiling water for not more than 2 minutes. This specification would imply that for periods up to 2 minutes, the treatment would have no influence on the ash content of the bones, whereas longer treatment may have an appreciable effect on the assay results. In view of this possibility, it was considered advisable to study the influence of varying periods of boiling of bones on their ash content.

In this investigation, 205 pairs of tibiae were used. After dissection and the removal of most of the tissue, the right tibiae were divided into 3 groups and immersed in boiling water for periods of 1, 2, and 4 minutes, respectively, following which the remainder of the adhering tissue was removed. The corresponding left tibiae were completely freed of tissue without boiling-water treatment.

A summary of the data and the significance of the mean differences between the per cent ash of the right and left bones are given in Table 3. These results indicate that the boiling treatment of 2 and 4 minutes as conducted in these experiments had a significant effect on the percentage ash of the tibiae. The 1 minute boiling period appeared to have had no appreciable effect.

In view of these results (Table 3) it seems advisable that the boiling time be kept within 1 minute. However, if a longer period is used, the

TABLE 3.—*Effect of boiling previous to extraction on percentage bone ash of chick tibiae*

TREATMENT	PAIRS OF BONES	MEAN BONE ASH	MEAN DIFFERENCE	t VALUE	PROBABILITY
		per cent	per cent		
None	64	35.24			
Boiled for 1 minute		35.21	0.03	0.272	>0.5
None	65	34.99			
Boiled for 2 minutes		34.65	0.34	3.92	<0.01
None	76	35.90			
Boiled for 4 minutes		35.05	0.85	7.50	<0.01

time of boiling should be carefully controlled in order that all the bones receive identical treatment.

#### IV. LENGTH OF ASHING PERIOD IN GROUP-ASH TECHNIC

Recently, Fritz and Halloran<sup>6</sup> have suggested that ashing at 850°C. for 1 hour may not be adequate to completely ash large bones, and reported, without presenting data, that in a few instances where larger bones were examined for ash content, a further loss of weight was found when the bones were reheated after 1 hour at 850°C. However, in this laboratory, it has been found that only a slight change in the weight of the ash occurred with further heating after incineration at 850°C. for 1 hour, even when large bones were being ashed and the muffle was fully charged.

In the present study, the bones were placed in a cold furnace, and the temperature was allowed to rise to 850°C., the time required for this procedure being approximately 1 hour. The bones were incinerated at 850°C. for 1 hour, and the ash was cooled in a desiccator and then weighed. Heating was continued at 850°C. for four successive  $\frac{1}{2}$ -hour intervals, and the ash weights were recorded after each heating.

Two experiments were conducted, one with a charge of 111 bones and the other a charge of 248 bones. The ashing was done in large crucibles containing 15–20 bones each. The inside dimensions of the muffle space were  $7\frac{1}{2} \times 5\frac{1}{4} \times 14$  inches.

The results of this study (Table 4) show clearly that continued heating for 2 hours after the initial incineration of 1 hour at 850°C. caused only a slight loss in ash weight, the loss in no case being greater than 1 per cent. It is evident from this investigation that an ashing period of 1 hour

<sup>6</sup> *Poultry Science*, 22, 314–322 (1943).

TABLE 4.—*Effect of length of ashing period on bone ash weight*

	BONE ASH AFTER 1 HOUR	NUMBER OF BONES	WEIGHT OF ASH AFTER HEATING AT 850°C.				
			1 HOUR*	1½ HOURS	2 HOURS	2½ HOURS	3 HOURS
	<i>per cent</i>		<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>grams</i>
<i>Experi- ment 1</i>	34.1	37	3.9461	3.9423	3.9415	3.9418	3.9398
	35.9	36	4.1629	4.1597	4.1595	4.1582	4.1574
	40.3	38	5.2899	5.2832	5.2799	5.2755	5.2749
Total		111	13.3989	13.3852	13.3809	13.3755	13.3721
<i>Experi- ment 2</i>	33.3	90	11.8115	11.7999	11.7891	11.7678	11.7564
	36.5	95	14.0626	14.0469	14.0331	14.0112	13.9918
	41.4	63	12.5152	12.5109	12.5036	12.4865	12.4754
Total		248	38.3893	38.3577	38.3258	38.2655	38.2236

\* Bones placed in cold muffle and temperature raised to 850°C. and held at this point for 1 hour.

at 850°C. as conducted in this laboratory gives comparative results for a wide variation in both the number and size of the bones that make up the charge of a muffle.

#### SUMMARY

From a detailed study of some of the steps in the treatment of chick tibiae relative to the determination of their percentage of ash, the following general observations have been made:

(1) Freezing of bones, as a method of storage, caused a significant decrease in the percentage of bone ash; storage in 95 per cent ethanol had no appreciable effect.

(2) Crushing of bones previous to solvent treatment had no significant effect on the amount of lipid material extracted as reflected in the percentage of ash.

(3) Immersion of bones in boiling water for periods longer than one minute caused a significant decrease in the percentage of bone ash.

(4) The ashing time of 1 hour at 850°C., as recommended by the A.O.A.C., appeared to be adequate for group ashing even when the muffle was fully charged.

#### ACKNOWLEDGMENT

The writers wish to acknowledge the technical assistance of S. J. Slinger and W. D. Graham with a part of this investigation.



## ASH DETERMINATIONS IN FOODS WITH AN ALKALINE BALANCE

### V. REACTIONS OF ALKALI CARBONATES WITH CALCIUM PHOSPHATES

By H. J. WICHMANN (Food Division,\* Food and Drug Administration,  
Federal Security Agency, Washington, D. C.)

Decomposition curves of the ash of a number of typical agricultural products are shown in the third paper of this series.<sup>1</sup> The decrease in weight of the ash of cocoa, milk, molasses, and orange juice above 500°–550°C. apparently can be ascribed only to a small degree to the decomposition of calcium carbonate. The writer ascribes it to reactions between alkali compounds and calcium or magnesium phosphates. The observations of Hillig<sup>2</sup> and the writer<sup>1</sup> that appreciable quantities of sodium and/or potassium are found in the water-insoluble ash of milk and cocoa also indicate that some hitherto unknown and unexpected reactions occur in the ashing process. Since ashing temperatures are well below the fusing temperatures of these compounds, such reactions must occur in the solid phase. While they may not, therefore, be strictly stoichiometric, they could nevertheless materially affect ash weights. To acquire more information regarding the reactions occurring during the ashing of a food product containing phosphates and excess alkali base, a series of heating experiments was made with various mixtures of calcium phosphates and sodium and potassium carbonates.

The writer has described the behavior of potassium carbonate on heating in air,<sup>3</sup> but before proceeding with the sodium carbonate experiments he considered it to be advisable to conduct a similar decomposition experiment with this salt. The results (Chart 1) indicate that the decomposition of sodium carbonate is like that of potassium carbonate except that the temperature of appreciable decomposition in air is about 100°C. higher. Therefore the maximum temperatures to which mixtures of potassium or sodium carbonates with alkaline earth phosphates can be heated without decomposition of the alkali carbonates are 700° and 800°C., respectively. These maximum temperatures, below the fusion temperatures of the alkali carbonates found in the reference books, were generally observed in experiments described in this paper. The maximum temperature of 800° C. in the sodium carbonate experiments caused some action on the platinum dishes and often sintered the reaction mixtures, though generally without actual fusion. Since fusion must be avoided in ash determinations the reactions investigated were limited as far as possible to those in the solid state.

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\* W. B. White, Chief.

<sup>1</sup> *This Journal*, 25, 478 (1942).

<sup>2</sup> *Ibid.*, 24, 744 (1941).

<sup>3</sup> *Ibid.*, 442.

Phosphorus exists in foods and agricultural products with an alkaline balance, both in organic combination and as inorganic phosphates. Ashing of such products, therefore, consists, in part at least, of a neutralization of the ortho acid phosphates or ortho phosphoric acid that may be present as such or produced in the process. The excess base is found in the ash as carbonates or oxides, depending on the nature of the base and on the ashing temperature. Pyro- or metaphosphates cannot be produced in ashes with excess base. To reduce this part of the ashing process to its

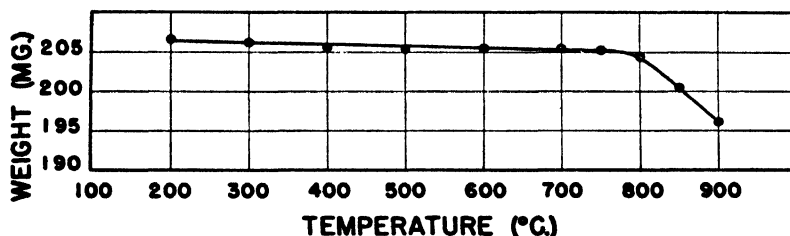


CHART. 1.—DECOMPOSITION CURVE OF  $\text{Na}_2\text{CO}_3$  IN AIR  
(Heating period at each point, 15 minutes)

simplest terms, experiments were made on the neutralization of the two acid orthophosphates of calcium by sodium or potassium carbonate at 200°–800°C. A precipitated basic calcium phosphate was also used in some experiments.

#### A. REACTIONS OF CALCIUM PHOSPHATES WITH SODIUM CARBONATE

##### 1. *Reaction of Monocalcium Phosphate with Sodium Carbonate*

Two hundred milligrams of monocalcium phosphate\* with one molecule of water of crystallization (93.01 per cent residue at 130°C. instead of a theoretical 92.85 per cent) was dissolved in water in a platinum dish, and sodium carbonate solutions containing known weights of the salt were then added to the phosphate. A gelatinous precipitate formed immediately and then flocculated on stirring and evaporation of the water on the steam bath. The residue was then heated for 15 minute intervals at increasing temperatures, usually beginning at 200°C. and ending at 800°C. in a temperature-controlled muffle furnace, according to previously described technic. On removal from the furnace the dishes were covered with an aluminum cover and cooled in an aluminum desiccator. After the last heating the residue was treated with 50 ml. of water, and the mixture was heated to incipient boiling for approximately 5 minutes, during which time the insoluble residue was crushed and disintegrated by a flat, rubber-tipped policeman. The mixture was then transferred quantitatively to a

\* Obtained from W. L. Hill, Division of Soil and Fertiliser Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

100 ml. volumetric flask with hot water, shaken well, and allowed to stand on the steam bath for approximately 10 minutes with intermittent shaking. The contents of the flask were then cooled to room temperature, made to mark, shaken well, and filtered on a dry filter, the filtrate being returned to the filter, if necessary, till a clear, or almost clear, filtrate resulted. Cloudy colloidal filtrates were sometimes obtained. Water-soluble phosphates were determined in the filtrate as phosphorus pentoxide by the volumetric phosphomolybdate method<sup>4</sup> and are expressed in the text as soluble phosphorus pentoxide. The water-insoluble residue was carefully transferred from the flask to the filter with hot water (100 ml.)

TABLE 1.—*Reactions of 200 mg. of  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  with  $\text{Na}_2\text{CO}_3$* 

	EXP. 1	EXP. 2	EXP. 3	EXP. 4
RATIO $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	.84	.95	.95	4.98
Maximum temp. of reaction (°C.)	800	800	800	800
Temp. of ignition of insol. ash (°C.)	800	800	800	800
Insol. ash (mg.)	88.5	95.8	88.8	84.0
Insol. CaO (mg.)	44.8	44.4	44.0	44.4
Insol. $\text{Na}_2\text{O}$ (mg.)	2.9	8.9	5.0	4.9
Insol. $\text{P}_2\text{O}_5$ (mg.)	38.4	40.2	37.0	32.8
Sum of insol. CaO, $\text{Na}_2\text{O}$ , & $\text{P}_2\text{O}_5$ (mg.)	86.1	93.5	86.0	82.1
Sol. $\text{P}_2\text{O}_5$ (mg.)	75.4	74.6	76.0	81.8
Sol. $\text{P}_2\text{O}_5$ /insol. $\text{P}_2\text{O}_5$	1.93	1.85	2.05	2.49
Insol. $\text{P}_2\text{O}_5$ /insol. CaO	.85	.90	.84	.74

(200 mg. of  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  contains 44.4 mg. of CaO and 112.6 mg. of  $\text{P}_2\text{O}_5$ .)

and then washed on the filter with about 100 ml. more hot water to remove all soluble salts. The water-insoluble residue and filter paper were ignited at approximately 550°C. and heated for 15 minutes at 800°C. When neutralization was made by potassium carbonate in later experiments the temperature of ignition was 700°C. These temperatures were selected originally because they mark the decomposition temperature in air of sodium and potassium carbonates. After being weighed, the water-insoluble residue, which will hereafter be referred to simply as insoluble ash, was dissolved in dilute hydrochloric acid and made up to 100 ml. in a volumetric flask. Calcium oxide,<sup>5</sup> sodium oxide,<sup>6</sup> and phosphorus pentoxide<sup>4</sup> were determined in aliquots of this solution. Whenever it is necessary to refer to a constituent of the water-insoluble ash in the body of this paper hereafter, it will be called insoluble sodium oxide etc. Curves were drawn on which the combined weights of the phosphate and sodium carbonate were plotted against temperatures from 200° to 800°C. It was thought possible to demonstrate the formation of definite compounds by the weight changes, and to correlate them with the composition of the

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1940, 22-23, 10-12.

<sup>5</sup> *Ibid.*, 339, 19.

<sup>6</sup> *Ibid.*, 130, 19.

ash. Since the ratio of alkali carbonate to alkaline earth phosphate might also have an effect on the reactions, two or more ratios were used. The results obtained are given in Table 1 and illustrated on Chart 2.

More or less definite weight equilibrium was attained, especially in Experiment 4 at 400°C., where it was maintained from 400° to about 650°C. As to the nature of the compound or compounds that are formed,

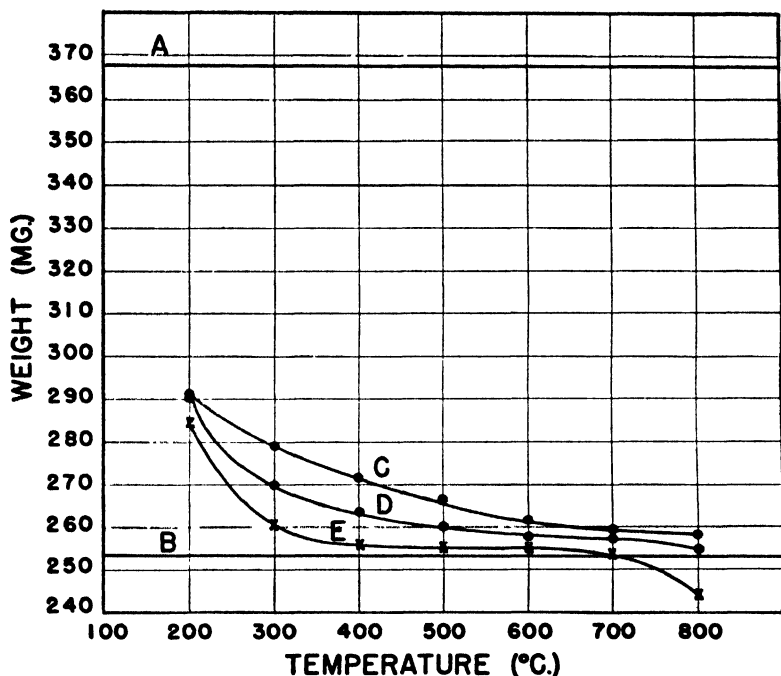
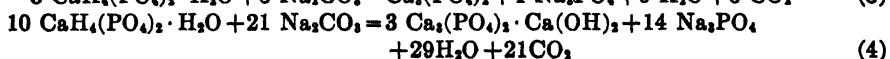
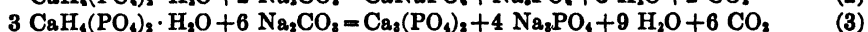
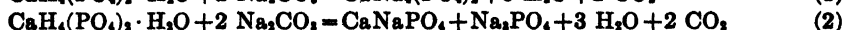
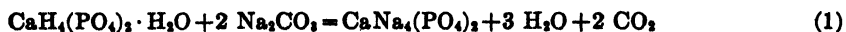


CHART. 2.—REACTION OF 200 MG. OF  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  WITH  $\text{Na}_2\text{CO}_3$   
(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.) (Exp. 1); B—Equilibrium weight on basis of Equation 4; C— $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = .84$  (Exp. 1); D— $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = .95$  (Exp. 2). Weights adjusted to Line A; E— $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 4.98$  (Exp. 4). Weights adjusted to Line A.

the conclusion might be drawn that the more or less horizontal parts of the curves mark the formation of a tertiary phosphate of some kind. Table 1 shows that all the insoluble ashes contained sodium, a condition somewhat contrary to the usually accepted rules concerning the solubility of sodium salts. It indicates that some unusual reactions may have occurred in these experiments. To visualize the nature of this neutralization at ashing temperatures, a number of possible equations were written and then subjected to criticism in the light of the data shown in Table 1 and Chart 2.



Equation 1 is inconsistent with the amount of soluble and insoluble phosphorus pentoxide shown in Table 1 and must, therefore, be considered untenable. Equation 2 should produce an equal division of the phosphorus between the soluble and insoluble ash. Table 1 shows that the division is far from equal and that the ratio of calcium to sodium in the insoluble ash is not that of the atomic weights. It is concluded, therefore, that Equation 2 is untenable so far as the principal reaction at 800°C. is concerned. It is possible that a small side reaction according to Equation 2 might account, in part, for the sodium in the insoluble ash. This possibility must be examined again.

Equation 3 demands that the soluble  $\text{P}_2\text{O}_5$ /insoluble  $\text{P}_2\text{O}_5$  ratio be equal to 2.0. Some of the ratios in Table 1 are near this value, but still the sodium found in the insoluble ash is not accounted for. As true tricalcium orthophosphate is not obtainable by wet methods of manufacture such as prevailed in the initial stages of the experiments, Equation 3 seems to be doubtful. Equation 4 requires a soluble  $\text{P}_2\text{O}_5$ /insoluble  $\text{P}_2\text{O}_5$  ratio of 14:6, or 2.33. The results in Table 1 fall on either side of this value and therefore deserve close attention. Basic tricalcium phosphate is also the usual form of the tertiary calcium phosphate produced by wet methods. If hydroxyapatite,  $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ ,\* and trisodium phosphate are the two non-volatile products produced by the reaction of sodium carbonate on monocalcium phosphate at 800°C., a water-insoluble ash determination should indicate the amount of hydroxyapatite, and its analysis should tally with its composition. The results of such determinations are also given in Table 1.

The finding of sodium in a mixture that is apparently mostly hydroxyapatite is not inconsistent with the experience of others, since it is known that sodium can be introduced in place of calcium into the inner structure of hydroxyapatite.<sup>7</sup> Hydroxyapatite has been described as a "trash-pile" that under favorable circumstances may contain other elements besides calcium and phosphorus. Therefore the sodium found in the insoluble ash might have entered it by a side reaction according to Equation 2 or by a base exchange imposed on the reaction expressed by Equation 4.

The ratio of sodium carbonate to the hydrated monocalcium phosphate in Equations 2 and 4 are .840 and .883, respectively, surprisingly close values. The first experiment was made with the former ratio. The experimental soluble  $\text{P}_2\text{O}_5$ /insoluble  $\text{P}_2\text{O}_5$  ratio of 1.93 is closer to the theoretical ratio of 2.33 demanded by Equation 4 than it is to 1.00, required by Equa-

\* Faust, Hendricks, and Reynolds, *Am. Mineral.*, 28, 356 (1943), state that the term "hydroxyapatite" is universally used in recent extensive German literature on the chemistry of this compound, but in the English literature since the time of Basset (1917) the term "hydroxyapatite" has been used.

<sup>7</sup> Hendricks and Jacob, private communication.

tion 2. The  $P_2O_5/CaO$  ratio of dicalcium phosphate, or calcium-sodium phosphate, is 1.26 and that of pure hydroxyapatite is 0.76. The experimental insoluble  $P_2O_5$ /insoluble  $CaO$  ratio of 0.85 is also closest to the hydroxyapatite ratio. Only 2.9 mg. of sodium, calculated as the oxide, was found in the insoluble ash, indicating that at best but a minute quantity of calcium-sodium phosphate could have been produced. However, the formation of a sodium-bearing hydroxyapatite could easily account for all the sodium found in the insoluble ash. If it is assumed that little or no calcium-sodium phosphate could have been produced, the distorted ratios might indicate the production of a small quantity of calcium-hydrogen phosphate from the monocalcium phosphate. Experiment 1 may therefore be considered in the main as an incompleting hydroxyapatite reaction.

More insoluble ash was obtained in Experiment 2 than in Experiment 1, and it also contained more sodium. The ratios, however, were rather close to those of the first experiment. These results may mean that an appreciable side reaction according to Equation 2 occurred at 800°C. in this experiment. Formation of calcium-sodium phosphate would disturb the ratios in the same way as would the formation of calcium-hydrogen phosphate and in addition increase the insoluble ash and its constituent.

Experiment 3 was conducted like Experiment 2 in all respects except that no weighings were made at temperatures below 800°C. The insoluble ash and the phosphorus data were very close to those found in Experiment 1, but the quantity of sodium in the insoluble ash was between the quantities found in Experiments 1 and 2. It cannot be stated solely from these results whether the sodium entered the insoluble ash by the mechanism of Equation 2, or as a displacement of some of the calcium in the hydroxyapatite. Possibly both reactions occurred. The ratios of Experiment 3 show an incomplete hydroxyapatite reaction even though there was a small excess of sodium carbonate present. The gelatinous and later flocculent nature of the precipitate formed may prevent complete reaction with the slight excess of sodium carbonate and leave phosphorus as calcium hydrogen phosphate, or in some instances, as calcium sodium phosphate.

In Experiment 4 there was an appreciable loss of weight at 800°C. Curve E is also located some distance below the other two curves, and it has a very noticeable horizontal portion between 400° and 650°C. At the end of the experiment the insoluble  $P_2O_5$  and the soluble  $P_2O_5$ /insoluble  $P_2O_5$  ratio were higher, and the insoluble  $P_2O_5$ /insoluble  $CaO$  ratio was lower than expected on the basis of Equation 4. This can only mean that in this experiment the reaction went beyond the first equilibrium, and was entering a second. No doubt the hydroxyapatite had begun to decompose at 800°C. with the production of more soluble  $P_2O_5$ , which in turn increased the soluble  $P_2O_5$ /insoluble  $P_2O_5$  ratio above 2.33 and decreased the insoluble  $P_2O_5$ /insoluble  $CaO$  ratio below 0.76. Other pertinent facts are (1) that the insoluble ash was more than the theoretical

79.9 mg. (basis of Equation 4); (2) that it contained about the same amount of sodium as was found in Experiment 3; and (3) that the weight of the calcium oxide, phosphorus pentoxide and sodium oxide found in all four experiments was less than the total weight of the insoluble ash.

If hydroxyapatite and trisodium phosphate are the non-volatile products of the reaction between monocalcium phosphate and excess sodium carbonate at 500°–700°C., the final calculable weights of the residue should coincide with the flat parts of the curves, especially in the case of curve E. The stoichiometric ratio of sodium carbonate to hydrated monocalcium phosphate in Equation 4 is 0.883; 200 mg. of the phosphate plus 176.6 mg. of sodium carbonate should produce a stoichiometric residue of 261.8 mg., and 114.8 mg. of water plus carbon dioxide should be volatilized. Subtracting 114.8 mg. from the combined weights of the phosphate and carbonate (horizontal line A) should give the actual weight of the residue if the reaction followed Equation 4 exactly. The horizontal line B in Chart 2 represents these calculations. It is obvious that the calculations locate this theoretical equilibrium a short but appreciable distance below the experimental data. Curve E is closer to line B than are the other two curves, which is understandable if curves C and D are considered as representing an incomplete reaction, or one complicated by a side reaction; it crosses line B at 700°C., where decomposition of hydroxyapatite probably begins. The reason for the slight discrepancy between experiment and theory as indicated by the position of curve E below 700°C. is still unexplained.

Equation 4 assumes that all the water and carbon dioxide formed in the hydroxyapatite reaction are volatilized. But it was shown in the fourth paper of this series<sup>8</sup> that precipitated basic calcium phosphate retained water of hydration tenaciously up to about 750°C. and that above that temperature the water of constitution of hydroxyapatite began to be driven off. Any water of hydration retained at temperatures below 700°C. could, at least in part, account for the upward shift of the experimental data, and its complete volatilization could make curve E approach line B at 700°C. It has been noted that analysis of the insoluble ash does not account for all of its weight. This is to be expected because part of the hydroxyl of hydroxyapatite,  $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ , is not determined. If the insoluble ash is heated to 800°C. or above, part of the loss of weight can be ascribed to loss of water of constitution. However, this kind of decomposition of hydroxyapatite differs from its decomposition by excess sodium carbonate although it contributes to the drop of curves like E between 700° and 800°C.

Another explanation for the upward shift in the experimental approach to equilibrium can be found in the behavior of calcium carbonate when heated in air. A partial displacement of calcium by sodium in the produc-

<sup>8</sup> *This Journal*, 25, 484 (1942).

tion of hydroxyapatite would leave the displaced calcium in the form of calcium carbonate up to temperatures of 600°–650°C. All the calcium derived from the monocalcium phosphate was found in the insoluble ash, which is consistent with the water insolubility of calcium carbonate. Further, its presence in the insoluble ash would produce the same insoluble  $P_2O_5$ /insoluble CaO ratio as does pure hydroxyapatite. However any carbon dioxide, combined with the calcium liberated from the monocalcium phosphate for which Equation 4 demands volatilization, could shift the experimental data upwards until the temperature became hot enough to decompose calcium carbonate when experiment and theory would be expected to coincide unless excess sodium carbonate affected further changes in the hydroxyapatite. The question as to whether the experimental shift is due to either of these causes or to a combination of both remains for further investigation. But it may be stated here that qualitative tests for carbon dioxide were usually positive on insoluble ashes obtained in this investigation, especially on those not highly heated. Whether all of this carbon dioxide came from calcium carbonate mixed with hydroxyapatite or whether part of it came from a carbonated hydroxyapatite or other carbonated calcium phosphates must also be determined later.

In the previous experiments the analysis of the ash was made after the final period of heating at 800°C. As the conditions existing between 200° and 800°C., especially with respect to the fixation of sodium in the insoluble ash and the exchange of phosphorus between calcium and sodium were of interest also, some analyses were made after each heating; 200 mg. of the monocalcium phosphate was partially dissolved in water in a platinum dish and treated with a sodium carbonate solution of such concentration as to produce the selected carbonate/phosphate ratio. The mixture was evaporated to dryness, the dish was placed in a cold muffle and heated to the temperature desired, and this temperature was maintained for 15 minutes. After the cooled dish had been weighed, insoluble ash, soluble phosphorus pentoxide, and insoluble sodium oxide were determined as before. The process was repeated for the different temperatures selected, and the data obtained were plotted on the same piece of cross-section paper. Chart 3 shows the results obtained with two different carbonate/phosphate ratios.

The weight-temperature curves of Chart 3 are similar to curves D and E of Chart 2, but when the insoluble ash vs. temperature, soluble  $P_2O_5$  vs. temperature, and the insoluble  $Na_2O$  vs. temperature plots are examined, the differences shown are somewhat surprising, especially in the experiments with the lower carbonate/phosphate ratio. Here something unusual was noticed during some of the hot water extractions. In the experiments made at 200°, 600°, and 800°C. the filtrate was clear, and the insoluble ash was readily filterable, but more or less colloidal and slowly



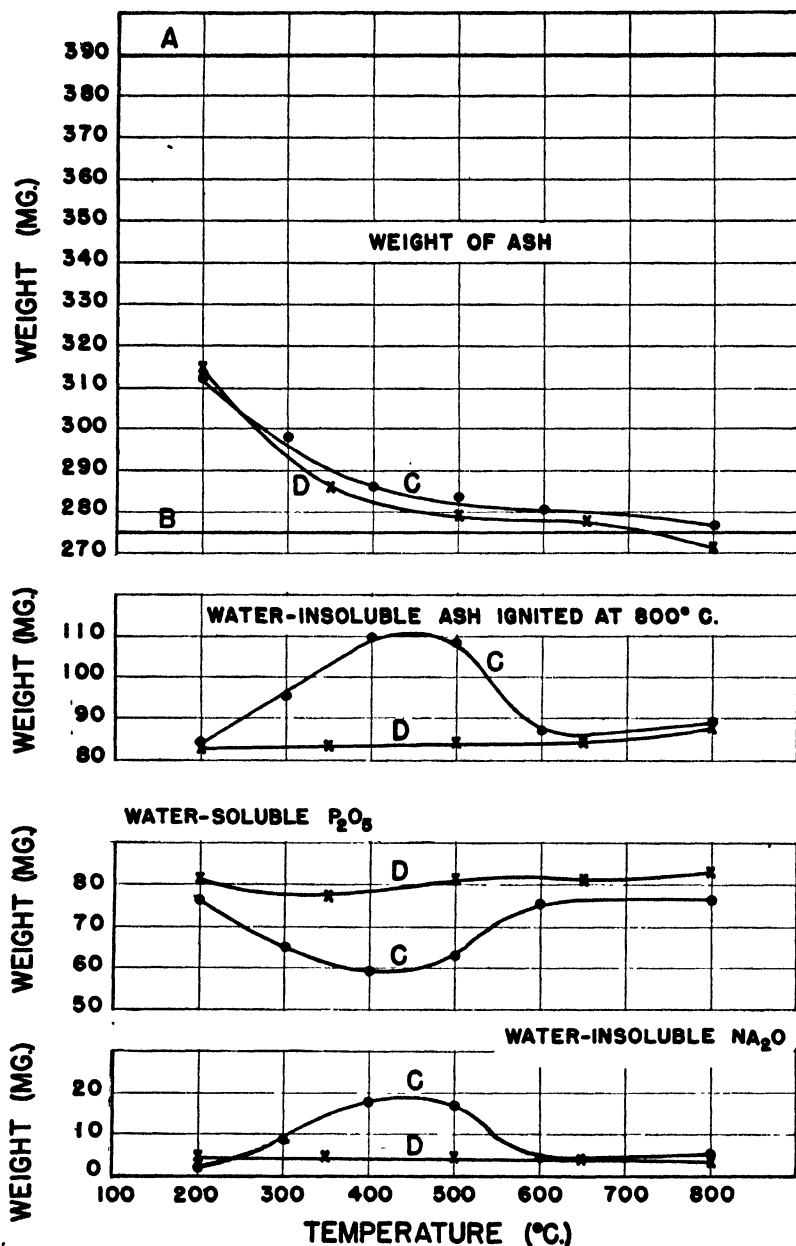


CHART. 3.—REACTION OF 200 MG. OF  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  WITH  $\text{Na}_2\text{CO}_3$   
(Heating period at each point, 15 minutes, and analysis made  
after each ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.) in experiments illustrated by Curve C; B—Equilibrium weight on basis of Equation 4; C— $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = .95$ ; D— $\text{Na}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 5.22$ , weights adjusted to Line A.

filtering filtrates were encountered in the experiments at 300°, 400°, and 500°C., which indicates a difference in the reactions. At 200°C. all the data for the two series of experiments very nearly coincide and again between 600° and 800°C. the curves are almost parallel and fairly close, but at 300°, 400°, and 500°C. the reaction introduced a greater proportion of sodium into the insoluble ash, and at the same time reduced the soluble phosphorus pentoxide. This condition must be due to the production of some calcium-sodium phosphate according to Equation 2. In the experiment at 400° C. the soluble  $P_2O_5$ /insoluble  $P_2O_5$  ratio was 1.20, which is not too far from 1.00, the value demanded by Equation 2. Partial hydrolysis of calcium-sodium phosphate during the hot water extraction would cause colloidal filtrates and tend to increase the ratio, and perhaps the production of hydroxyapatite was even less than the ratio of 1.20 would signify. Hydrolysis could also diminish the insoluble sodium. Perhaps a slightly lower carbonate-phosphate ratio would have eliminated the hydroxyapatite reaction entirely. A temperature above 200°C. is necessary to produce calcium-sodium phosphate under the conditions of alkalinity prevailing in these experiments, and the C curves show very definitely that the double phosphate is not stable above 600°C. under the conditions selected.

With the comparatively high carbonate-phosphate ratio of 5.22, the reaction is forced towards the hydroxyapatite equilibrium at all temperatures. The soluble phosphorus pentoxide is only slightly above normal from 200° to almost 800°C. The slight increase at 800°C. means a small decomposition of hydroxyapatite and corresponds with the dip in curve D below line B at the same temperature. The insoluble sodium oxide is not only approximately equal to the quantities given in Table 1, but it is almost uniform throughout the temperature range. These findings can only mean that the hydroxyapatite equilibrium predominated in these experiments. The hydroxyapatite produced contained a small amount of sodium introduced by a base exchange that seems to be affected very little by temperature. No doubt the C curve will approach the D curves as a limit as the carbonate-phosphate ratio is increased; on the other hand, the bulge in the C curve may broaden as the ratio is decreased. It was not believed necessary to investigate this point further, but attention is called to the differences in the slopes of the curves C and D between 500° and 600°C., the usual ashing temperature. Ashing temperature and carbonate/phosphate ratio exert no great influence on the weight of the "ash," but its composition is affected seriously. If similar conditions should be encountered in the ashing of an agricultural product, especially if neutralization with sodium carbonate is involved, the influence of these ratios on the analytical results (soluble phosphorus pentoxide, soluble or insoluble ash, and their alkalinities) should be marked.

## 2. Reaction of Dicalcium Phosphate with Sodium Carbonate

Approximately one gram of dicalcium phosphate\* was heated to about 900°C., and the loss of moisture on conversion into calcium pyrophosphate was determined. The loss of water was only 0.1 per cent greater than the theoretical value for anhydrous dicalcium phosphate, and for practical purposes this preparation may be considered to have the formula  $\text{CaHPO}_4$ . Two hundred milligrams of the product was treated with sodium carbonate solutions and then handled as described in the previous monocalcium phosphate experiments. The results obtained are shown in Table 2 and Chart 4.

TABLE 2.—Reaction of 200 mg. of  $\text{CaHPO}_4$ \* with  $\text{Na}_2\text{CO}_3$

	EXP. 1	EXP. 2	EXP. 3	EXP. 4
Ratio $\text{Na}_2\text{CO}_3/\text{CaHPO}_4$	.392 <sup>1</sup>	.392 <sup>2</sup>	.58	5.07
Maximum temp. of reaction (°C.)	800	800	800	800
Temp. of ignition of insol. ash (°C.)	800	800	800	800
Insol. ash (mg.)	180.0	189.0	153.2	149.7
Insol. $\text{CaO}$ (mg.)	81.2	82.0	82.0	82.8
Insol. $\text{Na}_2\text{O}$ (mg.)	12.6	21.4	4.2	9.3
Insol. $\text{P}_2\text{O}_5$ (mg.)	85.5	85.0	62.3	52.7
Sum of insol. $\text{CaO}$ , $\text{Na}_2\text{O}$ , & $\text{P}_2\text{O}_5$ (mg.)	179.3	188.4	148.3	144.8
Sol. $\text{P}_2\text{O}_5$ (mg.)	18.6	17.1	41.2	54.9
Sol. $\text{P}_2\text{O}_5$ /insol. $\text{P}_2\text{O}_5$	.22	.20	.66	1.04
Insol. $\text{P}_2\text{O}_5$ /insol. $\text{CaO}$	1.05	1.03	.76	.64

\* 200 mg. of  $\text{CaHPO}_4$  contains 82.4 mg. of  $\text{CaO}$  and 104.4 mg. of  $\text{P}_2\text{O}_5$ .

<sup>1</sup> Crust formed on the bottom of the dish and allowed to remain.

<sup>2</sup> Crust formed on the bottom of the dish, but was broken up by a rubber-tipped rod until the water-insoluble residue remained in a powdery and loose condition.

Two of the most probable equations involved in the reactions illustrated by the data of Table 2 and the curves of Chart 4 are:



The proportion of sodium carbonate to dicalcium phosphate in Equation 5 is as 1:0.389 and in Equation 6 as 1:0.467. Two hundred milligrams of dicalcium phosphate should produce 232.2 mg. of nonvolatile residue and 45.6 mg. of volatile matter according to Equation 5. The respective values for Equation 6 would be 244.0 and 49.4 mg. These calculations are illustrated by B and C in Chart 4. Line C is the same as B in Chart 2. Chart 4 again shows the upward shift in the experimental approach to the hydroxyapatite equilibrium discussed previously.

On evaporation of the mixtures of dicalcium phosphate and sodium carbonate solutions it was noted that the water-insoluble material crusted and stuck to the bottom of the dish. When this crust was broken up and

\* Obtained from W. L. Hill, Division of Soil and Fertiliser Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

pulverized with a rubber-tipped glass rod once or twice, the water-insoluble material remained in a powdery form and no longer encrusted on the bottom of the dish. This phenomenon seems important since it indicates a change in the character of the water-insoluble material, which in turn may mean a change in the character of the reactions that occurred in the dish. In Experiment 1 the crust was allowed to remain. At 800° C. the residual weight was considerably above lines B and C. In Experiments 1 and 2 the filtrate from the insoluble ash was more or less colloidal, and

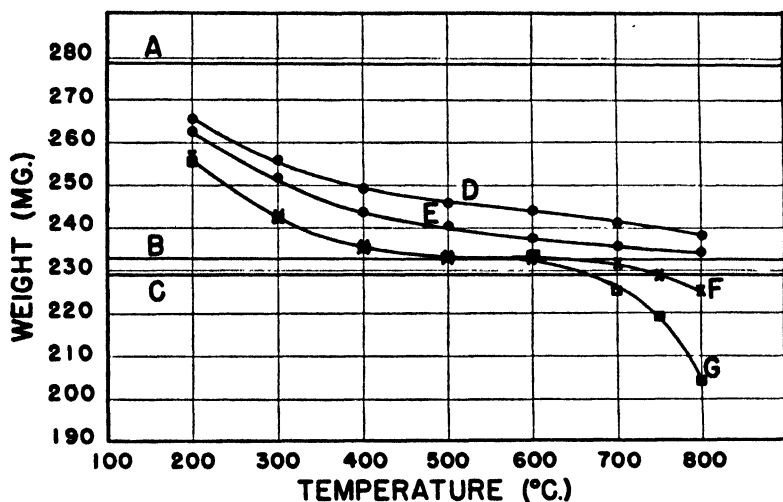


CHART 4.—REACTION OF 200 MG. OF  $\text{CaHPO}_4$  WITH  $\text{Na}_2\text{CO}_3$   
(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.), first 2 experiments; B—Equilibrium weight on basis of Equation 5; C—Equilibrium weight on basis of Equation 6; D— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=0.392$ , crust not broken (Exp. 1); E— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=0.392$ , crust broken (Exp. 2); F— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=0.58$ , crust broken (Exp. 3), weights adjusted to Line A; G— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=5.07$ , crust broken (Exp. 4), weights adjusted to Line A.

as in the previous section this probably means the production of calcium-sodium phosphate. The reaction illustrated by Equation 5 should produce no soluble  $\text{P}_2\text{O}_5$ . In Experiment 1 the soluble  $\text{P}_2\text{O}_5$  amounted to 18.6 mg., and the soluble  $\text{P}_2\text{O}_5$ /insoluble  $\text{P}_2\text{O}_5$  and the insoluble  $\text{P}_2\text{O}_5$ /insoluble  $\text{CaO}$  ratios were .22 and 1.05, respectively, instead of .66 and .76, the values for the hydroxyapatite equilibrium. A substantial amount of insoluble sodium oxide was found. These facts can only mean that both calcium-sodium phosphate and hydroxyapatite were produced in Experiment 1, although the soluble  $\text{P}_2\text{O}_5$  may not indicate the extent of the production of the latter; part of it may be due to hydrolysis of the double phosphate in the water extraction. Since curve D did not touch line B

at 800°C., it is concluded that not all the dicalcium phosphate had reacted with the sodium carbonate, due, no doubt, to the crust formation, which prevented complete reaction. Therefore, in this experiment part of the dicalcium phosphate remained intact, a portion reacted according to Equation 5, and a third portion produced hydroxyapatite.

In Experiment 2 the crust was carefully broken, and Table 2 shows that about the same amount of soluble  $P_2O_5$  was produced. But the increase of the insoluble ash was about the same as that of the insoluble sodium oxide. Curve E almost touches line B at 800°C. This is interpreted to indicate that about the same amount of hydroxyapatite was produced as in the previous experiment, but the proportion of the double phosphate in the reacting mixture at 800°C. was materially increased at the expense of residual dicalcium phosphate. Under such conditions a material change in the ratios could hardly be expected.

Experiment 3 was made with a carbonate/phosphate ratio of .58, which is in excess of that theoretically required for the hydroxyapatite reaction. The crust was also carefully broken during the evaporation of the mixture. Curve F crosses line C at 750°C. and ends at 800°C. slightly below it. The ratios obtained were exactly those required by Equation 6. The insoluble ash contained only 4.2 mg. of sodium oxide and the sum of the calcium oxide, sodium oxide, and phosphorus pentoxide determinations on the insoluble ash again was less than its weight. Curve F is again almost horizontal between 400° and 700°C. and located above line C. These results indicate that in this experiment conditions favorable to the hydroxyapatite equilibrium prevailed. The small amount of sodium oxide in the insoluble ash, as compared with the amount found in the first two experiments, indicates very limited side reactions. The observation relative to the upward shift of the experimental data between 400° and 700° in the previous section holds true in this experiment.

Experiment 4 (heavy excess of sodium carbonate) produced hydroxyapatite, which was decomposed at 800°C. to a considerable degree, as shown by the relatively large decrease in weight between 600° and 800°C. on Curve G and the distorted ratios in Table 2. The decomposition of hydroxyapatite with a comparatively large excess of sodium carbonate is no doubt greatly accelerated between 800°C. and fusion temperature when the phosphorus would be expected to become entirely water soluble. The sodium in the insoluble ash was more than twice the amount found in Experiment 3. Why this should be so is not apparent at this time. Some experiments were also made with dicalcium phosphate and sodium carbonate at temperatures between 200° and 800°C. under the same conditions. The results are shown in Charts 5 and 6. When the crust was not broken, even though the carbonate/phosphate ratio was higher than necessary for the theoretical hydroxyapatite equilibrium, some calcium-sodium phosphate was produced, as is indicated by the high insoluble ash, low soluble

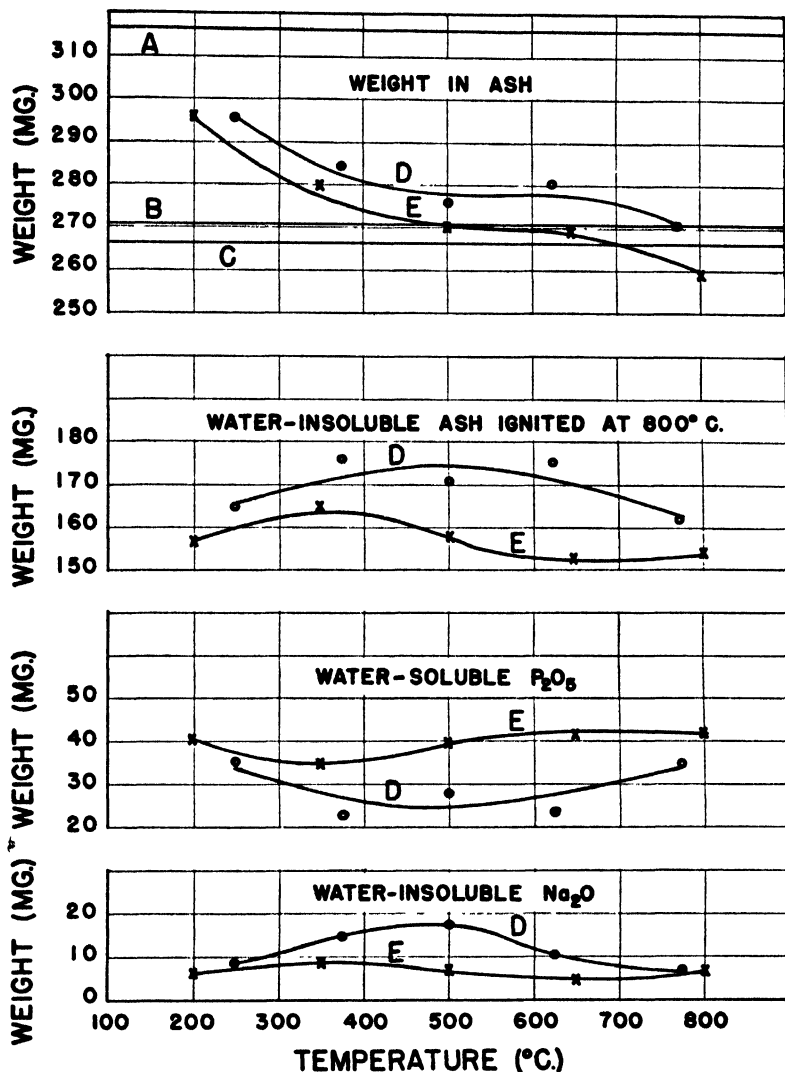


CHART 5.—REACTION OF 200 MG. OF  $\text{CaHPO}_4$  AND  $\text{Na}_2\text{CO}_3$   
(Heating period at each point, 15 minutes, and analysis made  
after each ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.) in experiment illustrated by D; B—Equilibrium weight on basis of Equation 5; C—Equilibrium weight on basis of Equation 6; D— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=0.58$ , reaction mixture crusted on bottom of dish; E— $\text{Na}_2\text{CO}_3/\text{CaHPO}_4=0.73$ , crust broken, weights adjusted to Line A.

phosphorus pentoxide, the high insoluble sodium oxide at certain temperatures, and possibly by the colloidal filtrate. There is a striking resemblance between the D curves of Chart 5 and the C curves of Chart 3. Again it

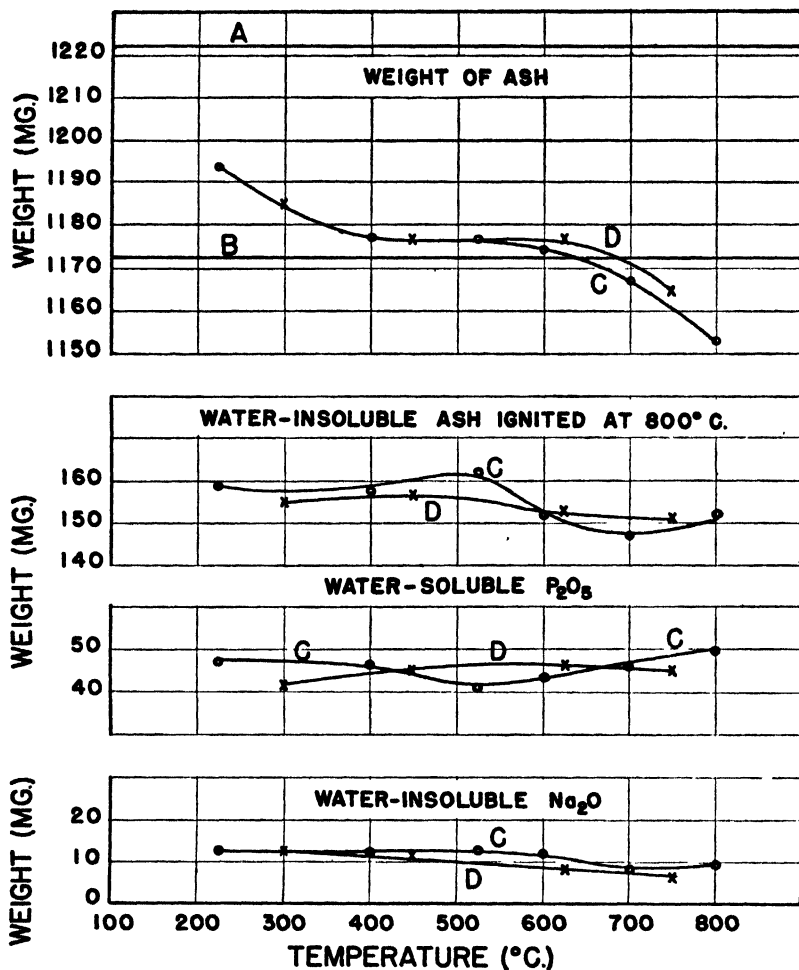


CHART 6.—REACTION OF 200 MG. OF  $CaHPO_4$  AND  $Na_2CO_3$   
(Heating period at each point, 15 minutes, and analysis made after  
each ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.);  
B—Equilibrium weight on basis of Equation 6; C— $Na_2CO_3/CaHPO_4=5.11$ , crust  
broken; D— $Na_2CO_3/CaHPO_4=5.11$ , crust not broken.

takes more than 200°C. to produce the double phosphate, and it is not stable above 600°C. in the presence of more than the theoretical amount of sodium carbonate. The erratic points on the D curves (the insoluble sodium oxide-temperature plot excepted) are due, no doubt, to variations in the proportions of the double phosphate, residual dicalcium phosphate, and hydroxyapatite of the crusted material. It was impossible to keep all conditions uniform.

The E curves illustrate experiments made with the crust carefully

broken up and with the carbonate/phosphate ratio high enough to force the reaction towards the hydroxyapatite equilibrium. The soluble phosphorus pentoxide data at 200°C. and again between 500° and 800°C. are consistent with Equation 6. The drop at 350°C. signifies, in the light of the information given by the D curves, that a small quantity of calcium-sodium phosphate might have been produced at this temperature. This supposition is confirmed by corresponding increases in the other plots, although the increase in the insoluble sodium oxide-temperature plot might ordinarily be considered merely an analytical error. The points on the E curves are more regular than those on the D curves, which is undoubtedly due to the breaking up of the crust. The sodium entered the hydroxyapatite molecule at comparatively low temperatures and remained almost constant throughout the temperature range.

Chart 6 illustrates two experiments with the same high carbonate/phosphate ratio, the C curves showing data obtained when the crust was broken and the D curves when it was not. When the ratio is high enough, the reaction seems to turn from the double phosphate towards the hydroxyapatite equilibrium, crust or no crust.

It has been suggested that the bulges in the curves could contract or expand according to the value of the carbonate/phosphate ratio, but that the curves approached the straight line of the hydroxyapatite equilibrium as a limit. The same tendency is found in the dicalcium phosphate experiments. The small bulge at 350°C. in the E curves of Chart 5 indicates a double phosphate remnant. A higher or lower temperature forced the reaction toward the hydroxyapatite equilibrium with the ratio of .73. Apparently a ratio as high as 5.11 is not needed to produce hydroxyapatite provided the crust is broken. The curves of Chart 6 show the early entrance of sodium into the hydroxyapatite at about the level shown in Experiment 4 of Table 2, and that increased temperature has but little effect. The significance on curve C of the soluble  $P_2O_5$ -temperature plot of the minimum at 525°C. (which corresponds to the theoretical value according to Equation 6) is not apparent. However the high point at 800°C. is quite understandable because it indicates some decomposition of hydroxyapatite by the excess alkali. The curves of Chart 6, in connection with those of Chart 5, show very definitely that when the excess of alkali is great enough the two principal nonvolatile products are hydroxyapatite and trisodium phosphate and that under such conditions there is practically no side reaction. But even when the reaction is forced into a predominantly hydroxyapatite equilibrium, the experimental data are above the theoretical requirements up to 650°-700°C.

### *3. Reaction of Precipitated Basic Calcium Phosphate with Sodium Carbonate*

The precipitated basic calcium phosphate used in these experiments was from the same sample described in Paper 4 of this series.<sup>8</sup> Its composition



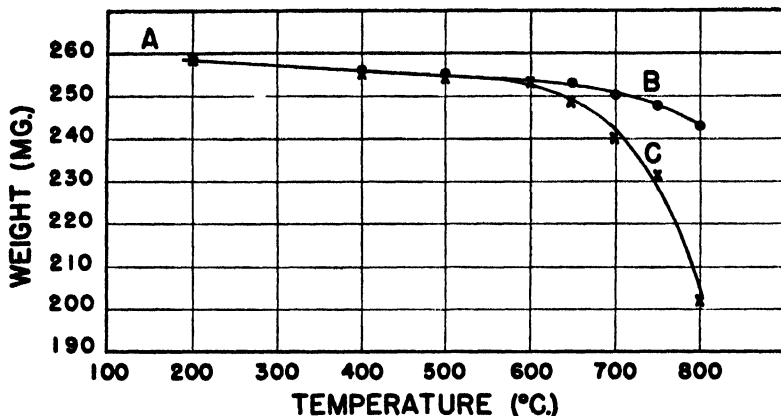


CHART 7.—REACTION OF 200 MG. OF PRECIPITATED BASIC CALCIUM PHOSPHATE WITH  $\text{Na}_2\text{CO}_3$

(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.) (Exp. 1); B— $\text{Na}_2\text{CO}_3$ /basic calcium phosphate = .30 (Exp. 1); C— $\text{Na}_2\text{CO}_3$ /basic calcium phosphate = 3.55 (Exp. 3), weights adjusted to Line A.

was about half-way between tricalcium phosphate and hydroxyapatite. Some further reaction between it and sodium carbonate is therefore possible. Decomposition of the hydroxyapatite with excess sodium carbonate at higher temperatures is also probable in the light of previous findings. The results obtained in these experiments are shown in Table 3 and Chart 7.

TABLE 3.—Reaction of 200 mg. of precipitated basic calcium phosphate\* with  $\text{Na}_2\text{CO}_3$

	EXP. 1	EXP. 2	EXP. 3
Ratio $\text{Na}_2\text{CO}_3$ /prec. basic Calcium phosphate	.30	.55	3.55
Maximum temp. of reaction (°C.)	800	800	800
Temp. of ignition of insol. ash (°C.)	800	700 & 800	800
Insol. ash (mg.)	192.2	192.8 at 700°C. 190.5 at 800°C.	181.3
Insol. CaO (mg.)	105.2	107.6	106.4
Insol. $\text{Na}_2\text{O}$ (mg.)	2.9	7.1	6.9
Insol. $\text{P}_2\text{O}_5$ (mg.)	81.8	75.6	61.0
Sum of insol. CaO, $\text{Na}_2\text{O}$ , $\text{P}_2\text{O}_5$ (mg.)	189.9	190.3	174.3
Sol. $\text{P}_2\text{O}_5$ (mg.)	5.8	10.2	25.7
Sol. $\text{P}_2\text{O}_5$ /insol. $\text{P}_2\text{O}_5$	.07	.13	.42
Insol. $\text{P}_2\text{O}_5$ /insol. CaO	.77	.70	.51

\* 200 mg. of the precipitated basic calcium phosphate contains 84.8 mg. of  $\text{P}_2\text{O}_5$  and 106.6 mg. of CaO.

The noteworthy information in Table 3 is the fact that again sodium is found in the insoluble ash, even when there was not much reaction, as

measured by the small quantity of phosphorus pentoxide made soluble. The precipitated basic calcium phosphate seems to be quite sensitive to the decomposing action of excess sodium carbonate at the higher temperatures. This is particularly striking in Experiment 3.

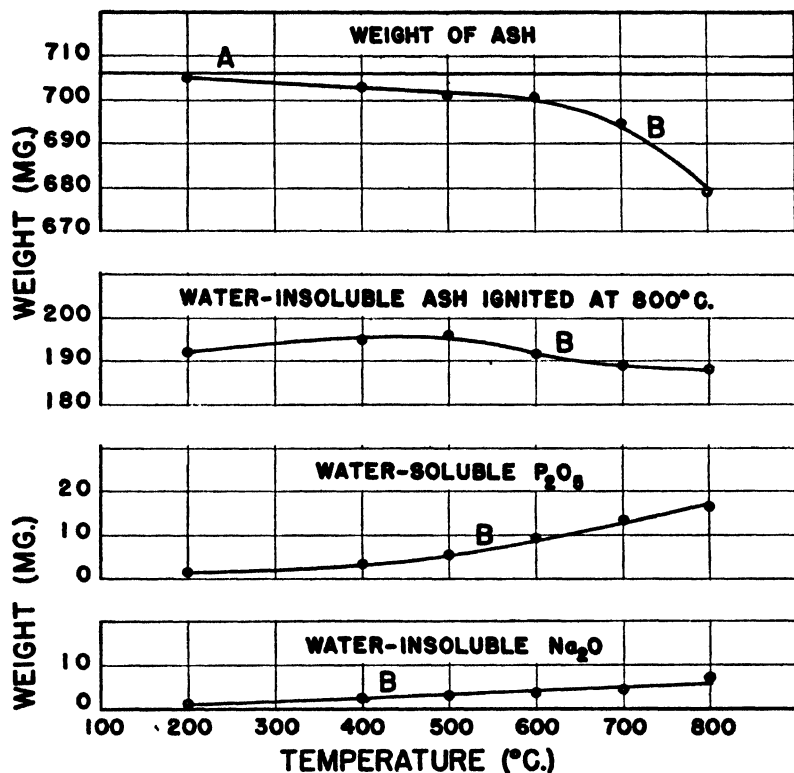


CHART 8.—REACTION OF 200 MG. OF PRECIPITATED BASIC CALCIUM PHOSPHATE WITH  $Na_2CO_3$

(Heating period at each point, 15 minutes, and analysis made after ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.);  
B— $Na_2CO_3$ /basic calcium phosphate = 2.53.

Chart 8 illustrates the reactions of precipitated basic calcium phosphate with sodium carbonate at intermediate temperatures. The soluble phosphorus pentoxide and the insoluble sodium oxide increased with rising temperatures, gradually at first and then more rapidly. This might be predicted by the shape of the curves in Chart 7.

The water-soluble alkalinity and soluble phosphorus pentoxide determinations on agricultural ashes containing calcium, phosphorus, and sodium must be functions of the reactions that the ingredients of the ash under-

went during the ashing. These experiments show that the reactions can be quite variable and that they depend primarily on the ratios of the alkalis to the alkaline earth phosphates and on the temperature. It is expected that the information obtained so far will be useful when applied later to the ashing of actual food samples.

## B. REACTIONS OF CALCIUM PHOSPHATE WITH POTASSIUM CARBONATE

### 1. Reaction of Monocalcium Phosphate with Potassium Carbonate

The experiments with monocalcium phosphate and potassium carbonate were conducted as described in Series A experiments, except

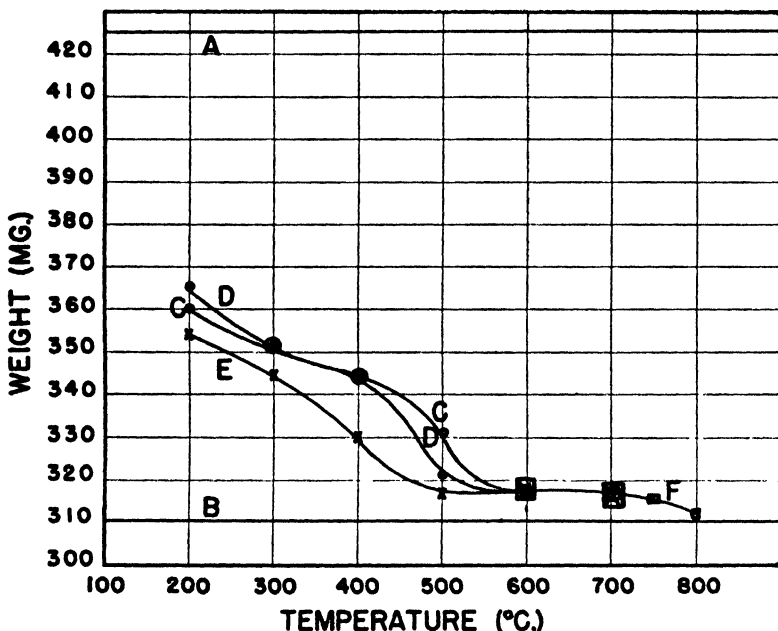


CHART 9.—REACTION OF 200 MG. OF  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  WITH  $\text{K}_2\text{CO}_3$   
(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.) (Exp. 1); B—Equilibrium weight on basis of Equation 8; C— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 1.126$  (Exp. 1); D— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 1.31$  (Exp. 2), weights adjusted to Line A; E— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 5.01$  (Exp. 3), weights adjusted to Line A; F— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 5.05$  (Exp. 4), weights adjusted to Line A.

that a maximum temperature of 700°C. (incipient decomposition of potassium carbonate) was employed in three of the experiments and 800°C. was deliberately chosen in another. Potassium oxide was determined in insoluble ashes by the triple nitrite method of Wilcox.<sup>9</sup> The results are shown on Chart 9 and in Table 4.

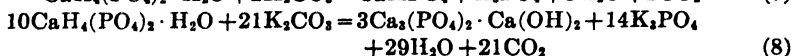
<sup>9</sup> *Ind. Eng. Chem., Anal. Ed.*, 9, 136 (1937).

TABLE 4.—*Reaction of 200 mg. of  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  with  $\text{K}_2\text{CO}_3$* 

	EXP. 1	EXP. 2	EXP. 3	EXP. 4
Ratio $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	1.126	1.31	5.01	5.05
Maximum temp. of reaction (°C.)	700	700	700	800
Temp. of ignition of insol. ash (°C.)	700	700 & 800	700	700 & 800
Insol. ash (mg.)	83.4	84.1 at 700 83.0 at 800	81.7	83.4 at 700 80.8 at 800
Insol. CaO (mg.)	44.0	44.8	44.8	45.6
Insol. $\text{K}_2\text{O}$ (mg.)	Trace*	Trace*	Trace*	Trace*
Insol. $\text{P}_2\text{O}_5$ (mg.)	35.3	36.3	34.1	34.7
Sum of insol. CaO, $\text{K}_2\text{O}$ , $\text{P}_2\text{O}_5$ (mg.)	79.3	81.1	78.9	80.3
Sol. $\text{P}_2\text{O}_5$ (mg.)	78.6	79.0	80.6	80.9
Sol. $\text{P}_2\text{O}_5$ /insol. $\text{P}_2\text{O}_5$	2.22	2.17	2.36	2.33
Insol. $\text{P}_2\text{O}_5$ /insol. CaO	.81	.81	.76	.76

\* A trace means a visible precipitate of triple nitrite that corresponds to not more than 0.5 mg. of  $\text{K}_2\text{O}$ .

If the reactions between monocalcium phosphate and potassium carbonate are analogous to the previously described sodium carbonate reactions the two most probable equations illustrating the reactions are as follows:



The stoichiometric relationships between hydrated monocalcium phosphate and potassium carbonate according to these equations are as 1:1.096 and 1:1.15, respectively. These reactions should produce 306.5 and 315.3 mg. of non-volatile residue and 112.7 and 114.7 mg. of volatile water plus carbon dioxide, respectively. The calculations for Equation 8 are expressed by line B on Chart 9, below the flat part of the experimental curves.

The data in Table 4 are similar to those in Table 1 with the striking exception that only traces of potassium were found in the insoluble ash when the reaction was carried to 700° or 800°C. This indicates that potassium cannot enter the hydroxyapatite molecule or produce a double phosphate at 800°C. as does sodium under certain conditions. In the first two experiments, the soluble  $\text{P}_2\text{O}_5$  and the ratios indicate that reaction 8 was not quite completed. In Experiments 3 and 4 the ratios were normal for the hydroxyapatite reaction. If Experiments 1 and 2 are not complete reactions, curves C and D of Chart 9 might be expected to be slightly above curves E and F at 600°–700°C. just as curves C and D are in Chart 2, and it is not apparent why they are not. If the ratios are accurate, Experiments 3 and 4 apparently produced pure hydroxyapatite with no potassium. The formation of calcium carbonate by a base exchange at 500°–700°C., such as occurred in the sodium carbonate experiments, seems improbable. However, the fact that the curves are still above the theoretical hydroxyapatite equilibrium represented by line B is as yet unexplainable.

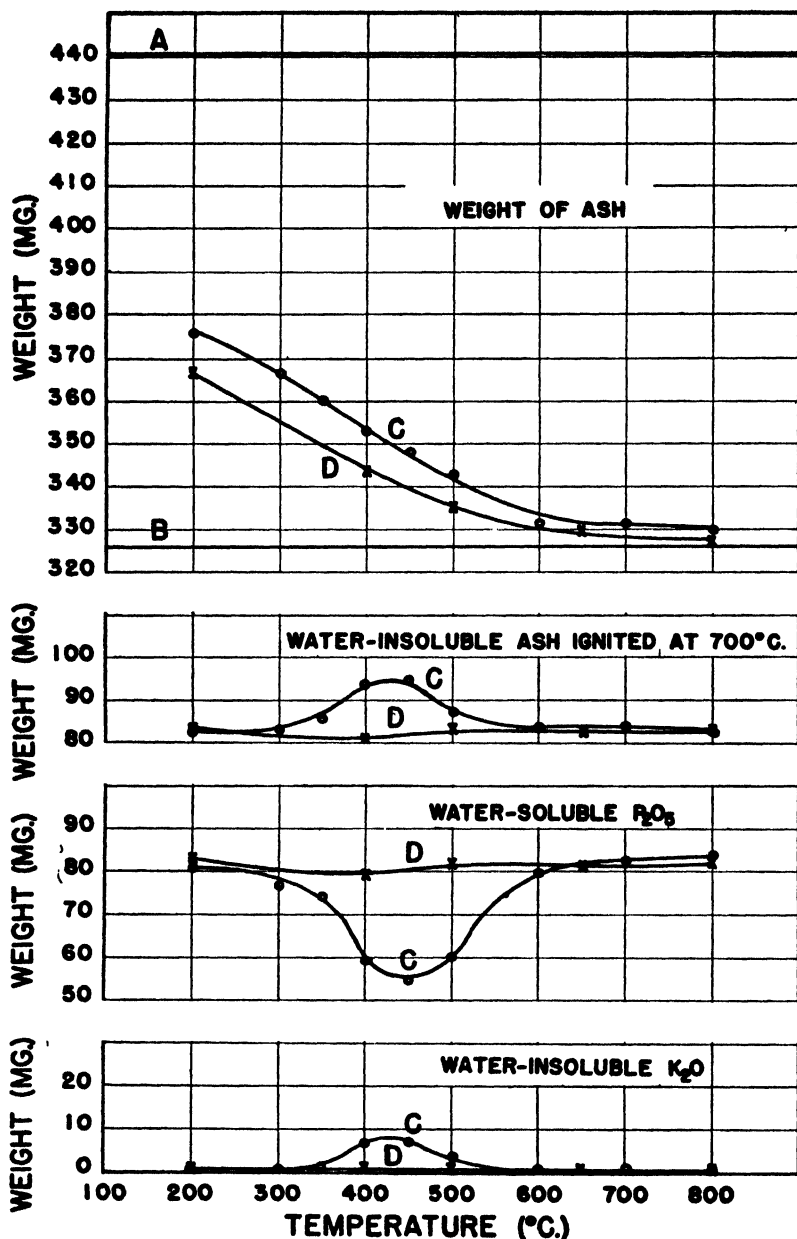


CHART 10.—REACTION OF 200 MG. OF  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  WITH  $\text{K}_2\text{CO}_3$   
(Heating period at each point, 15 minutes, and analysis made after  
each ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.) in experiments illustrated by C; B—Equilibrium weight on basis of Equation 8; C— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 1.203$ ; D— $\text{K}_2\text{CO}_3/\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O} = 4.85$ , weights adjusted to Line A.

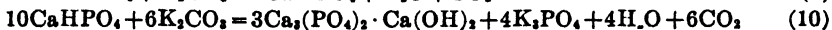
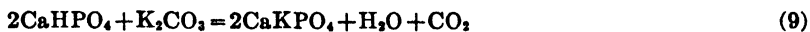
Chart 10 illustrates the experiments made with monocalcium phosphate and two concentrations of potassium carbonate, between 200° and 800°C. The curves are qualitatively if not quantitatively similar to those in Chart 3. The weight-temperature plot, unlike the curves in Chart 9, does show a small difference between curves C and D from 600° to 800°C. The C curves of Chart 10 show that in the experiments conducted at 350°–600°C., potassium entered the insoluble ash, probably by virtue of a side reaction (Equation 7) just as sodium (Equation 2) did in the experiments illustrated by Chart 3. The soluble  $P_2O_5$ /insoluble  $P_2O_5$  and the insoluble  $P_2O_5$ /insoluble CaO ratios for the experiment at 400°C. were 1.44 and 0.95, respectively, indicating the presence of a considerable proportion of the double phosphate. The soluble and insoluble phosphorus pentoxide added up to 100.4 mg. of the 112.6 mg. originally present in the 200 mg. of monocalcium phosphate. It thus appears that 12.2 mg. of phosphorus pentoxide must have been lost during the washing of the insoluble ash on the filter. Perhaps some potassium was likewise extracted because of hydrolysis of the calcium-potassium phosphate and this may, in part, explain the smaller quantities of insoluble potassium found in these experiments as compared with the insoluble sodium found in the previous experiments. Little or no insoluble potassium was found in experiments conducted at temperatures less than 300°C., or at 600°C. or over. The double phosphate of calcium and potassium, like the analogous sodium compound, is not stable at high temperatures in the presence of excess alkali, nor does it form at low temperatures.

The D curves on Chart 10 show that the side reaction (Equation 7) does not occur at any temperature if the carbonate-phosphate ratio is high enough, and that in such cases it produces hydroxyapatite containing little or no potassium. In none of these experiments was as much as 1 mg. of potassium oxide found in the insoluble ash.

## 2. Reactions of Dicalcium Phosphate with Potassium Carbonate

The experiments with dicalcium phosphate and potassium carbonate were conducted as were those with sodium carbonate. Crusting of the water-insoluble residue in the platinum dish was again observed and handled in the same manner as before. The results of these experiments are given in Table 5 and on Chart 11.

The two equations that best illustrate the data in Table 5 and Chart 11 are:



The proportions between dicalcium phosphate and potassium carbonate in the above equations are 1:0.507 and 1:0.609. Reaction 9 should produce 255.8 mg. of nonvolatile residue and 45.6 mg. of water and carbon dioxide. Equation 10 requires 272.4 and 49.4 mg., respectively.

TABLE 5.—Reaction of 200 mg. of  $\text{CaHPO}_4$  with  $\text{K}_2\text{CO}_3$ 

	EXP. 1	EXP. 2	EXP. 3	EXP. 4	EXP. 5
Ratio $\text{K}_2\text{CO}_3/\text{CaHPO}_4$	.512	.65	.84	1.07	5.11
Maximum temp. of re- action ( $^{\circ}\text{C}.$ )	750	750	700	750	750
Temp. of ignition of insol. ash ( $^{\circ}\text{C}.$ )	700	700	700 & 800	700	700 & 800
Insol. ash (mg.)	179.7	160.0	157.0 at 700 155.5 at 800	155.0	146.3 at 700 143.8 at 800
Insol. $\text{CaO}$ (mg.)	82.4	81.6	83.2	83.5	84.2
Insol. $\text{K}_2\text{O}$ (mg.)	17.6	5.4	3.0	1.3	Trace*
Insol. $\text{P}_2\text{O}_5$ (mg.)	73.4	67.2	62.9	63.5	57.0
Sum of insol. $\text{CaO}$ , $\text{K}_2\text{O}$ , $\text{P}_2\text{O}_5$ (mg.)	173.4	154.2	149.1	148.3	142.5
Sol. $\text{P}_2\text{O}_5$ (mg.)	24.8	35.5	40.3	43.0	49.3
Sol. $\text{P}_2\text{O}_5$ /insol. $\text{P}_2\text{O}_5$	.33	.52	.64	.67	.86
Insol. $\text{P}_2\text{O}_5$ /insol. $\text{CaO}$	.89	.82	.76	.76	.67

\* Trace if precipitate is visible but not more than .5 mg. of  $\text{K}_2\text{O}$ .

The curves of Chart 11 are quite similar to those of Chart 4. In curve F the equilibrium point between  $500^{\circ}$  and  $650^{\circ}\text{C}.$  is above the theoretical value illustrated by line C, as in all previous experiments. The carbonate/phosphate ratio of .65 in Experiment 2 is above the value demanded by Equation 10 but curve E did not intersect line C at  $750^{\circ}\text{C}.$ ; it was even

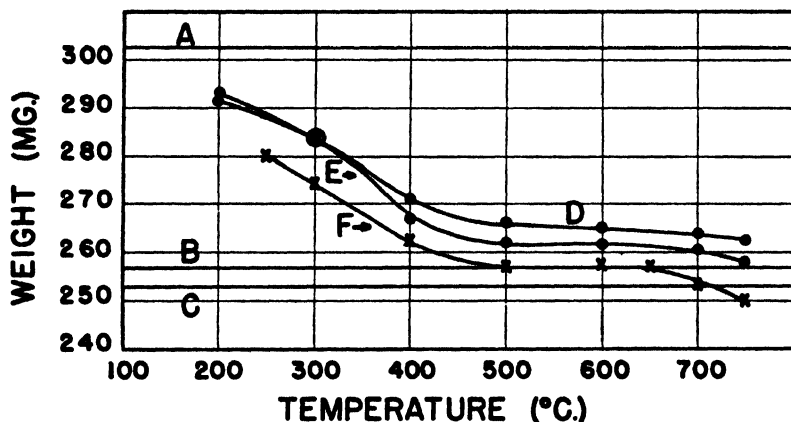


CHART 11.—REACTION OF 200 MG. OF  $\text{CaHPO}_4$  WITH  $\text{K}_2\text{CO}_3$   
(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.) (Exp. 1); B—Equilibrium weight on basis of Equation 9; C—Equilibrium weight on basis of Equation 10; D— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=0.512$ , crust broken (Exp.1); E— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=0.650$ , crust broken (Exp. 2), weights adjusted to Line A; F— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=5.11$ , crust broken (Exp. 5), weights adjusted to Line A.

slightly above line B, which is drawn at the weight required by Equation 9. The data in Table 5 may explain its location.

Table 5 shows that the insoluble potassium decreases with ascending carbonate/phosphate ratios. Equation 9 would require zero soluble phosphorus pentoxide. This table also shows that an increasing amount of phosphorus became soluble with ascending ratios, and these facts show that an increasing proportion of hydroxyapatite was produced at 750°C. at the expense of the double phosphate as the carbonate/phosphate ratio was increased. In Experiment 5 only a trace of insoluble potassium was found, which is consistent with a previous statement that potassium is rarely if ever found in hydroxyapatite produced at 800°C. in the presence of sufficient excess potassium carbonate. The insoluble potassium found in Experiment 1 and in much smaller quantities in Experiments 2, 3, and 4 probably was introduced by a side reaction illustrated by Equation 9, but the possibility of a partial base exchange similar to the mechanics of the sodium introduction into hydroxyapatite is not discounted. The progressions in the soluble  $P_2O_5$ /insoluble  $P_2O_5$  ratios and the retrogressions in the insoluble  $P_2O_5$ /insoluble CaO ratios should be noted. In Experiments 2, 3, and 4 the remnants of insoluble potassium progressively decreased, and the ratios indicate that the hydroxyapatite was the predominant reaction. In Experiment 5 the hydroxyapatite had begun to decompose, as was also the case in the dicalcium phosphate-sodium carbonate experiments at high ratios.

On Chart 12 are illustrated the results of experiments conducted at temperatures between 200° and 700°C. The curves of this chart should be compared with those of Charts 5 and 6. At a carbonate/phosphate ratio of 0.98, and without breaking of the crust, the double phosphate was produced at all temperatures tried except 200°C. This is shown by the F curves. At 200°C. no insoluble potassium was found. In connection with the low soluble phosphorus pentoxide this must mean that only a portion of the dicalcium phosphate reacted with the potassium carbonate to form hydroxyapatite. When the crust was broken (but the same ratio retained) small but quite determinable quantities of potassium were found in the insoluble ash at 200°, 350°, and 500°C., less at 600°C., and almost none at 700°C. Similar quantities were found when the carbonate/phosphate ratio was raised to 4.85. The fact that little or no insoluble potassium was found in the experiments at 700°C. is consistent with the data in Table 5. Why there should be quite appreciable quantities of insoluble potassium at lower temperatures, its nature, and its disappearance when the temperature reaches 700°C. are unexplained phenomena at this time. Curve E in the soluble phosphorus pentoxide-temperature plot shows that the soluble phosphorus pentoxide is even a little higher than theory requires for the hydroxyapatite equilibrium, and that it persists throughout the temperature range. There is little possibility, therefore, that the insoluble potas-



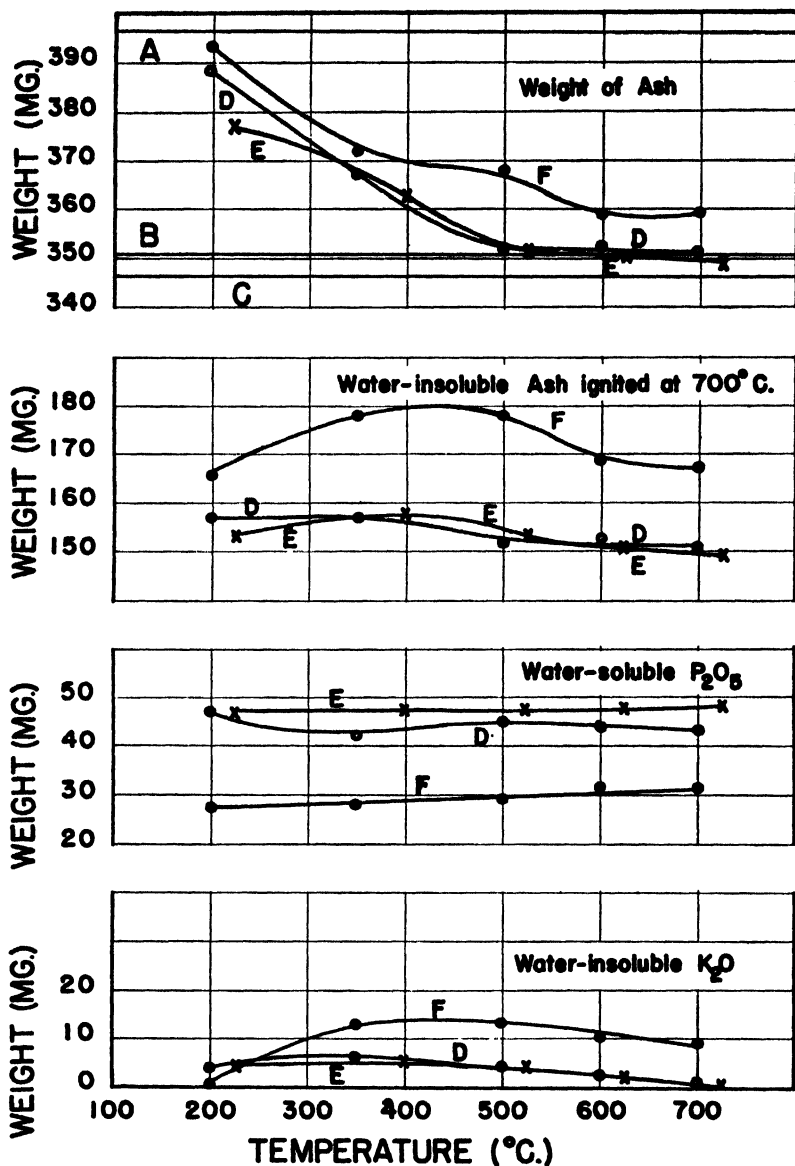


CHART 12.—REACTION OF 200 MG. OF  $\text{CaHPO}_4$  WITH  $\text{K}_2\text{CO}_3$   
(Heating period at each point, 15 minutes, and analysis made after each ash had been weighed)

A—Reference Line—combined weight of phosphate and carbonate (mg.) in experiments illustrated by D; B—Equilibrium weight on basis of Equation 9; C—Equilibrium weight on basis of Equation 10; D— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=0.98$ , crust broken; E— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=4.85$ , crust broken, weights adjusted to Line A; F— $\text{K}_2\text{CO}_3/\text{CaHPO}_4=0.98$ , reaction mixture crusted on bottom of dish.

sium was derived from calcium-potassium phosphate. As it seemed to be possible that during these experiments potassium was forced into the hydroxyapatite molecule at moderate temperatures, a further experiment was made at 450°C. with a carbonate/phosphate ratio of over 5.0. The crust was carefully pulverized and a more complete analysis was made. The insoluble potassium oxide amounted to 4.6, the soluble phosphorus pentoxide to 48.5, and the insoluble phosphorus pentoxide to 56.2 mg. The soluble  $P_2O_5$ /insoluble  $P_2O_5$  and the insoluble  $P_2O_5$ /insoluble CaO ratios were 0.86 and 0.68, respectively, which were almost exactly the same results as were found in Experiment 5 at 750°C. (Table 5). However the insoluble ashes ignited at 700° were 151.7 and 146.3 mg. The difference of 5.4 mg. is almost accounted for by the insoluble potassium oxide found in one experiment but not in the other. The sum of the results of the insoluble phosphorus pentoxide, calcium oxide, and potassium oxide determinations for insoluble ash was 142.8, which left 8.9 mg. unaccounted for (water and carbon dioxide). This evidence points towards the presence of a potassium-containing hydroxyapatite mixed with an equivalent amount of calcium oxide in the insoluble ash ignited at 700°C., but the data in the insoluble potassium oxide-temperature plot on Chart 12 further show that apparently the potassium-containing hydroxyapatite is not a stable compound in the presence of excess potassium carbonate and ashing temperatures over 500°–600°C., because the insoluble potassium decreases with increasing temperature until it disappears at about 700°C. This is also indicated by the results of Experiment 5 of Table 5. The insoluble potassium data shown in Experiments 1, 2, 3, and 4 (Table 5) were previously shown to be due to the presence of calcium-potassium phosphate in the insoluble ash. It is apparent, therefore, that insoluble potassium should be found in agricultural ashes, but the nature of its combination depends upon temperature and the carbonate/phosphate ratio.

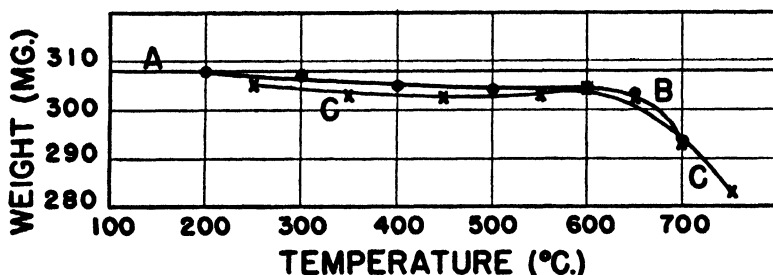
### *3. Reaction of Precipitated Basic Calcium Phosphate with Potassium Carbonate*

These experiments were conducted in the same manner as were those in A, 3, and the results are shown in Table 6 and Chart 13.

Excess potassium carbonate produces about the same degree of decomposition in basic precipitated calcium phosphate as does sodium carbonate at the same temperature, but no significant quantity of insoluble potassium was found. That seems to be about the only difference between these experiments and those shown on Chart 7. Between 200° and 700°C. the results are about the same as those shown on Chart 8 (sodium carbonate experiments), except that the insoluble potassium did not exceed 1 mg. at any temperature tried.

TABLE 6.—Reaction of 200 mg. of precipitated basic calcium phosphate with  $K_2CO_3$ 

	EXP. 1	EXP. 2
Ratio of $K_2CO_3$ /prec. basic calcium phosphate	.54	3.84
Maximum temp. of reaction ( $^{\circ}C$ .)	700	750
Temp. of ignition of insol. ash	700	700
Insol. ash (mg.)	191.5	189.7
Insol. CaO (mg.)	106.0	106.0
Insol. $K_2O$ (mg.)	Trace	Trace
Insol. $P_2O_5$ (mg.)	80.2	74.0
Sum of insol. CaO & $P_2O_5$ (mg.)	186.2	180.0
Sol. $P_2O_5$ (mg.)	6.7	13.8
Sol. $P_2O_5$ /insol. $P_2O_5$	0.08	0.18
Insol. $P_2O_5$ /insol. CaO	0.75	0.70

CHART 13.—REACTION OF 200 MG. OF PRECIPITATED BASIC CALCIUM PHOSPHATE WITH  $K_2CO_3$ 

(Heating period at each point, 15 minutes)

A—Reference Line—combined weight of phosphate and carbonate (mg.) (Exp. 1); B— $K_2CO_3$ /basic calcium phosphate = 0.54 (Exp. 1); C— $K_2CO_3$ /basic calcium phosphate = 3.84 (Exp. 2), weights adjusted to Line A.

#### DIFFERENCE BETWEEN EXPERIMENTAL DATA AND THEORETICAL EQUILIBRIUM

It has been repeatedly noted in previous sections that the experimental data frequently did not coincide with theory. Further investigation of this point is carried out in this section.

The non-volatile compounds remaining after calcium phosphates have been heated with alkali carbonates under ashing conditions are, or may be, tribasic alkali phosphates; calcium-alkali double phosphates under certain conditions; calcium hydroxyapatite containing sodium and, in a few instances, potassium; calcium carbonate probably equivalent to the alkali that entered the hydroxyapatite molecule; and perhaps other carbonates, the existence of which has not yet been considered. If the double phosphates, instead of hydroxyapatite, are produced to any appreciable degree, it can be readily understood why these experimental data should be above those demanded by theory, on the basis of the

hydroxyapatite reaction. It is because the combined weight of volatilizable water and carbon dioxide is less. However, the retention of water by hydroxyapatite, or of carbon dioxide by calcium carbonate or other carbonates, produced in the reaction, could also account for the difference. A series of experiments was therefore undertaken in an attempt to test this hypothesis.

First the water-retaining properties of trisodium and tripotassium orthophosphates were studied. Various quantities of pure grades of these

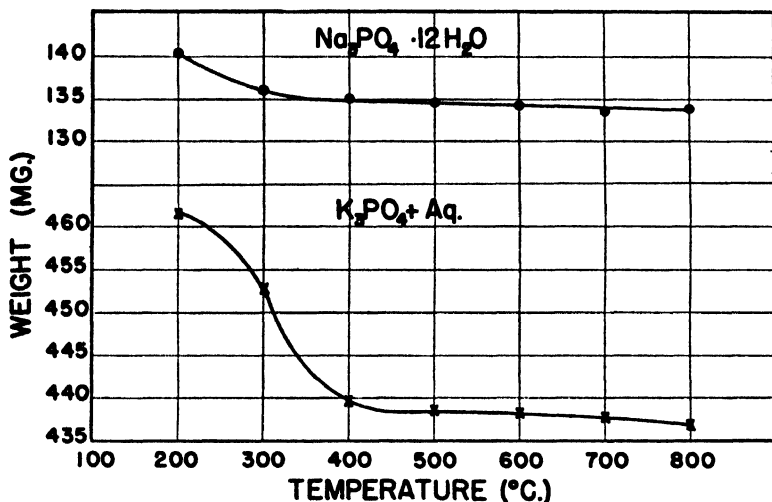


CHART 14.—LOSS OF WATER FROM TRIBASIC SODIUM AND POTASSIUM ORTHOPHOSPHATES (Heating period at each point, 15 minutes)

salts were heated in platinum dishes fitted with aluminum covers for 15-minute intervals at increasing temperatures, as in previous experiments. The results are shown on Chart 14. The curves show that the two salts become almost anhydrous at 400°C. and that the loss between 400° and 800°C. is less than one per cent of the weight of the salts. The upward shift of the experimental data could be due only to a very minor degree to the retention of water by these tribasic phosphates formed in the reaction. The reason for the shift must therefore be sought in the insoluble ash.

Three insoluble ashes were produced from mono- and dicalcium phosphate by the action of sodium carbonate and extraction with hot water in such a manner that the production of hydroxyapatite was favored in two instances and that of calcium-sodium phosphate in the third. The insoluble ash was washed from the filter back into the platinum dish, the water was evaporated on the steam bath, and the residue was heated for 15 minute intervals at increasing temperatures. The results are shown by the curves on Chart 15.

Curves A and C show the results obtained with insoluble ashes derived

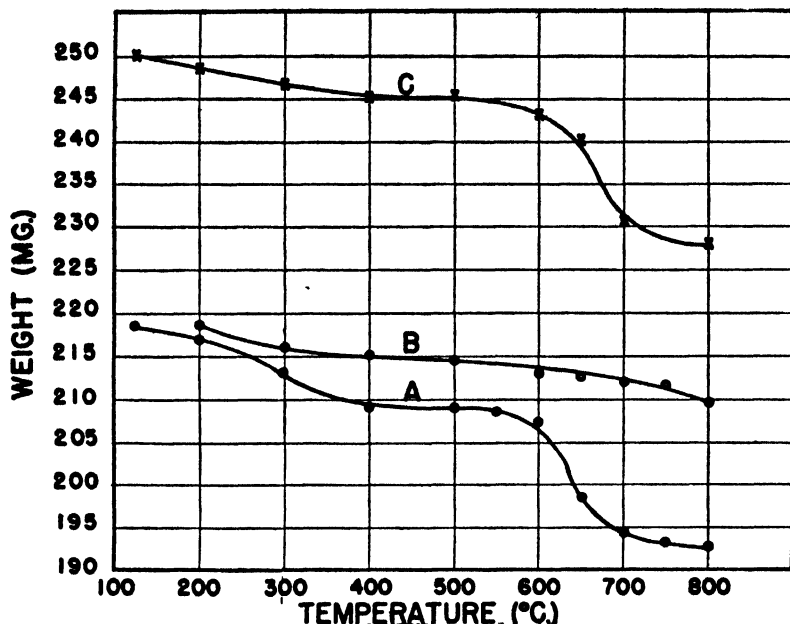


CHART 15.—LOSS OF WEIGHT OF WATER-INSOLUBLE ASHES DERIVED FROM THE REACTION OF CALCIUM PHOSPHATES AND SODIUM CARBONATE AT 500°C. WITH INCREASE OF TEMPERATURE

(Heating period at each point, 15 minutes)

A—Water-insoluble ash from  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  and  $\text{Na}_2\text{CO}_3$  at 500°C. Carbonate-phosphate ratio = 6.0, conditions favorable for production of sodium containing hydroxyapatite, ash contained 6.1 mg. of  $\text{Na}_2\text{O}$ ; B—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{Na}_2\text{CO}_3$  at 500°C. and carbonate-phosphate ratio of 0.42, crust well pulverized, conditions favorable for production of  $\text{CaNaPO}_4$ ; 250 mg. of  $\text{CaHPO}_4$  produced 21.1 mg. of water-soluble  $\text{P}_2\text{O}_5$ , water-insoluble ash contained 21.1 mg. of  $\text{Na}_2\text{O}$ ; C—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{Na}_2\text{CO}_3$  at 500°C. and carbonate-phosphate ratio over 5.0, crust well pulverized, conditions favorable for production of sodium containing hydroxyapatite. Ash contained 13.1 mg. of  $\text{Na}_2\text{O}$ .

from mono- and dicalcium phosphates, respectively, under conditions favoring the formation of hydroxyapatite. Sharp breaks occur between 600° and 700°C., which previous studies have shown to be the temperatures between which calcium carbonate decomposes most rapidly in air.<sup>3</sup> They, therefore, corroborate the theory advanced in previous sections that the shift in the experimental data was due to unvolatilized carbon dioxide combined as calcium carbonate with the lime set free when sodium entered the hydroxyapatite molecule. The decomposition of the calcium carbonate corresponds to the drop between 600° and 700°C. in curve E on Chart 2 and in curves F and G on Chart 4, where they cross the line representing the hydroxyapatite equilibrium. The break in curve C is not so sharp as the one in curve A, or that for pure calcium carbonate.<sup>3</sup> This may

mean that this insoluble ash contained something besides sodium-containing hydroxyapatite and calcium carbonate.

Curve B shows what happens when an insoluble ash, consisting largely if not entirely of the double phosphate ( $\text{CaNaPO}_4$ ), is heated. Curve B is in distinct contrast to curves A and C in that the loss of weight of the water-insoluble ash between  $500^\circ$  and  $800^\circ\text{C}$ . is small and gradual instead of sudden and considerable. This difference in the behavior of insoluble ash towards heat reflects the difference in composition. The production of 21.1 mg. of soluble phosphorus pentoxide in the formation of this insoluble ash indicates that about one-sixth of it should be hydroxyapatite (which should contain some sodium according to previous experience). But the lack of a noticeable break between  $600^\circ$  and  $700^\circ\text{C}$ . precludes the admixture in the insoluble ash of any appreciable amount of calcium carbonate equivalent to the sodium that presumably entered the hydroxyapatite. This is somewhat surprising and requires further investigation.

The losses of weight of insoluble ashes registered by curves A and C are believed to be due principally to the volatilization of carbon dioxide from the decomposition of calcium carbonate, but part of the loss could be due to water volatilized from the hydroxyapatite.<sup>8</sup> The approximately 3 mg. lost by the insoluble ash illustrated by Curve B between  $500^\circ$  and  $750^\circ\text{C}$ . could be either carbon dioxide or water. A better insight into the nature of these losses might be possible if the insoluble ash could be subjected to controlled temperatures under conditions that allow the collection and weighing of semimicro amounts of water and carbon dioxide volatilized at the different temperatures. Unfortunately no apparatus for such experiments was available. The nearest substitute was to heat the insoluble ashes in platinum dishes at the desired temperatures and then determine the percentage of carbon dioxide in the residues with a micro Van Slyke apparatus. Therefore a series of insoluble ashes was prepared similar to those illustrated in Chart 15, but instead of heating them at temperatures from  $500^\circ$  to  $800^\circ\text{C}$ . or over and then weighing the residues, the percentage of carbon dioxide was determined in 10–15 mg. quantities of the residue after each heating. The results are illustrated by the curves on Chart 16.

The curves on Chart 16 show clearly that all the insoluble ashes derived from the reaction of mono- and dicalcium phosphates with sodium carbonate contain residual carbon dioxide, but it is also evident that the carbon dioxide must exist in at least three combinations, some of which resist the action of heat more than others. Curves A, C, and D illustrate the percentages of carbon dioxide in insoluble ashes characterized by the presence of sodium-containing hydroxyapatite. These curves are similar in shape to Curves A and C of Chart 15, which show the weight losses of the same kind of insoluble ashes. The curves on Chart 16 indicate that the percentage of carbon dioxide in the insoluble ashes drops at the same temperature and has the same lag in the break as the weight losses shown on

Chart 15. Therefore, it may be concluded that the weight losses shown by curves A and C on Chart 15 are due mostly to the volatilization of carbon dioxide from calcium carbonate and not to that of water. In addition these curves (Chart 16) show that the insoluble ashes contain another kind of carbonate, one that can retain at least some of its carbon dioxide up to 800°C. in contrast to the almost complete volatilization of the gas from calcium carbonate at 700°C. The presence of a heat-resisting carbon-

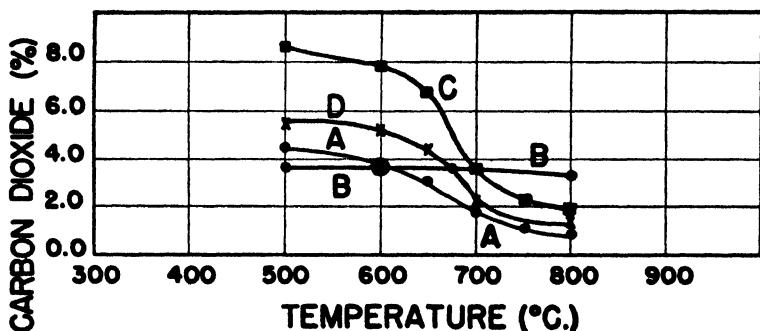


CHART 16.—CO<sub>2</sub> IN WATER-INSOLUBLE ASHES (%), DERIVED FROM REACTION OF CALCIUM PHOSPHATE AND SODIUM CARBONATE AT 500°C. AT DIFFERENT TEMPERATURES

(Heating period at each point, 15 minutes)

A—Water-insoluble ash from  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  and  $\text{Na}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio = 3.0, conditions favorable for production of sodium containing hydroxyapatite; B—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{Na}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio = 0.40, crust well pulverized, conditions favorable for production of  $\text{CaNaPO}_4$ , 250 mg. of  $\text{CaHPO}_4$  produced 22.0 mg. of water-soluble  $\text{P}_2\text{O}_5$ ; C—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{Na}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio = 6.0, crust well pulverized, conditions favorable for production of sodium containing hydroxyapatite; D—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{Na}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio = 1.0.

ate possibly explains why the break in Curve C of Chart 16 is not so sharp between 600° and 700°C. as it should be on the basis of decomposition of calcium carbonate alone. But what is the nature of the carbonate that is more heat resisting than calcium carbonate? Hendricks, Hill, Jacob, and Jefferson,<sup>10</sup> and Franck, Bredig, and Frank<sup>11</sup> have called attention to the existence of the carbonate-apatites. The writer has now reason to believe that similar compounds exist, to some extent at least, in the insoluble ashes produced in these experiments. Apparently increased sodium carbonate in the reaction mixture causes increased amounts of these carbonate-apatites. The writer understands<sup>12</sup> that the carbon dioxide of carbonated apatite is substituted in part for the  $\text{PO}_4$  radical in the inner

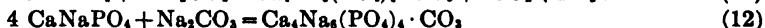
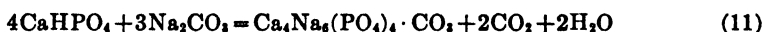
<sup>10</sup> *Ind. Eng. Chem.*, 23, 1413 (1931).

<sup>11</sup> *Z. anorg. Chem.*, 230, 2 (1936).

<sup>12</sup> W. L. Hill, private communication.

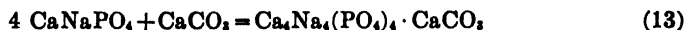
part of the hydroxyapatite molecule,  $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$ . It is perhaps immaterial in a study of ashes and ash methods to develop the exact location in the molecule of this carbon dioxide. It should be sufficient to show that carbonated apatites can exist in certain insoluble ashes, and that this carbon dioxide, together with that from calcium carbonate, probably accounts for the major part of the upward shift of the experimental curves toward the theoretical equilibrium curves.

Curve B of Chart 16 shows that this insoluble ash contained a moderate quantity of carbon dioxide that is not volatilized between  $500^\circ$  and  $800^\circ\text{C}$ . The small loss of weight between  $400^\circ$  and  $800^\circ\text{C}$ . shown by Curve B of Chart 15 must, therefore, have been due mostly to a volatilization of water and not carbon dioxide. Previous work (Table 2) has shown that a carbonate-phosphate ratio of 0.4 produced an insoluble ash consisting largely, it was believed at the time, of a double calcium-sodium phosphate. Evidently on the basis of Curve B, heat-resisting carbon dioxide must now be introduced into the double phosphate molecule in some manner. Franck, Bredig, and Frank<sup>11</sup> describe a quaternary compound, produced by them at  $1100^\circ$ – $1200^\circ\text{C}$ . from calcium phosphates and sodium carbonate in an atmosphere of carbon dioxide and designated by them as rhenanite. The formulas are:



This compound may perhaps be described more exactly by the formula:  $4\text{CaNaPO}_4 \cdot \text{Na}_2\text{CO}_3$ , that is, a basic calcium-sodium phosphate. This investigation suggests that with the proper ratio of dicalcium phosphate and sodium carbonate, rhenanite or mixtures of rhenanite and calcium-sodium phosphate may be produced at ashing temperatures.

The B curves on both charts show no definite breaks between  $600^\circ$  and  $700^\circ\text{C}$ ., indicating the absence of calcium carbonate in the insoluble ash, but the formation of 21 and 22 mg. of soluble phosphorus pentoxide in the production of these insoluble ashes needs some explanation since the production of neither the rhenanite nor calcium-sodium phosphate would be expected to involve the formation of soluble phosphates. Perhaps the soluble phosphorus pentoxide shows the formation of a limited quantity of sodium-containing hydroxyapatite, but the equivalent calcium carbonate might have reacted at  $500^\circ\text{C}$ . with some of the calcium-sodium phosphate to form a species of rhenanite, according to the Franck, Bredig, and Frank equation for sodium rhenanite; thus:



This suggestion does not imply that all the carbon dioxide found in the insoluble ash entered it by the mechanism of the above equation (compare Equations 11 and 12), but it might account for the soluble phosphorus pentoxide results and for the absence of any calcium carbonate.



The insoluble ashes represented by the B curves of Charts 15 and 16 may be a mixture of hydroxyapatite, calcium-sodium phosphate, and sodium and calcium rhenanite. To "unravel" such a mixture would require complicated separations and analyses and perhaps the service of unavailable X-ray apparatus. At present it can only be said that the insoluble ashes

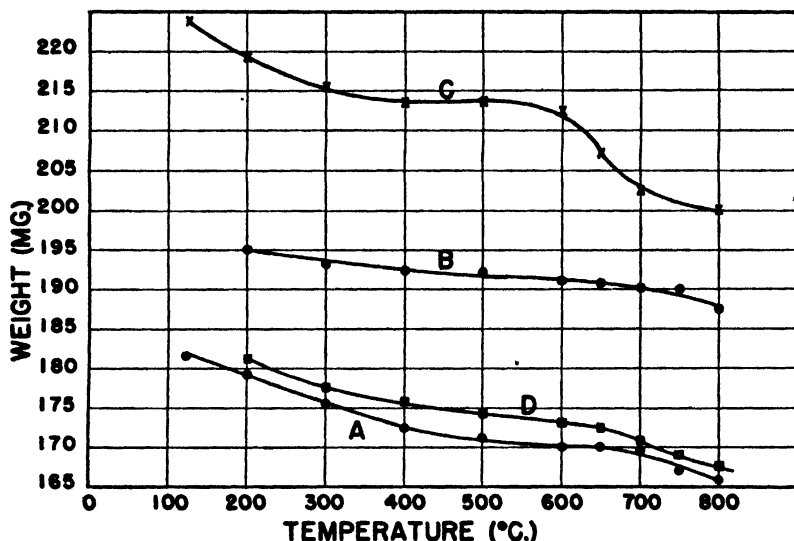


CHART 17.—LOSS OF WEIGHT OF WATER-INSOLUBLE ASHES DERIVED FROM REACTION OF CALCIUM PHOSPHATES AND  $K_2CO_3$  AT 500° AND 700°C. WITH INCREASE OF TEMPERATURE

(Heating period at each point, 15 minutes)

A—Water-insoluble ash from  $CaH_4(PO_4)_2 \cdot H_2O$  and  $K_2CO_3$  at 500°C. with carbonate-phosphate ratio over 6.0, conditions favorable for production of hydroxyapatite, water-insoluble ash contained less than 1 mg. of  $K_2O$ ; B—Water-insoluble ash from  $CaHPO_4$  and  $K_2CO_3$  at 500°C. with carbonate-phosphate ratio of 0.512, conditions favorable for production of  $CaKPO_4$ , 250 mg. of  $CaHPO_4$  produced 23.4 mg. of water-soluble  $P_2O_5$ , water-insoluble ash contained 11.8 mg. of  $K_2O$ ; C—Water-insoluble ash from  $CaHPO_4$  and  $K_2CO_3$  at 500°C. with carbonate-phosphate ratio over 5.0, crust well pulverized, conditions favorable for production of potassium-bearing hydroxyapatite (see Fig. 12), water-insoluble ash contained 7.6 mg. of  $K_2O$ ; D—Water-insoluble ash from  $CaHPO_4$  and  $K_2CO_3$  at 700°C. with carbonate-phosphate ratio over 5.0, conditions favorable for production of hydroxyapatite without potassium (see Table 5 and Fig. 12), water-insoluble ash contained less than 1 mg. of  $K_2O$ .

derived from the reaction of sodium carbonate on calcium phosphates at ashing temperatures contain carbonates of diverse character that explain the differences between the experimental data and those theoretical equilibria that do not take these carbonates into account. The presence of incompletely decomposed carbonated apatites or rhenanites at 700° or 800°C. would also account for the previous incomplete analyses of insolu-

ble ashes ignited at the same temperatures, which did not include carbon dioxide determinations because a suitable semimicro method apparatus was not available at the time.

The weight losses of insoluble ashes derived from the reactions of potassium carbonate on calcium phosphates are shown by the curves on Chart 17. The evidence in Section B II first pointed towards the possibility of the production of potassium-containing hydroxyapatites under certain limiting conditions of temperatures and carbonate-phosphate ratios. Curve C, because of its similarity to Curve C of Chart 15, seems to confirm this evidence. The 7.6 mg. of insoluble potassium oxide, as well as the break in Curve C between 600° and 700°C., indicates, according to previous experience, the presence of admixed calcium carbonate in the insoluble ash approximately equivalent to the potassium that apparently entered the hydroxyapatite molecule. Previous experiments also indicated that the potassium-containing hydroxyapatite was not stable at 700°C. in the presence of excess potassium carbonate. Curve D illustrates the weight losses of insoluble ash derived from a reaction mixture heated to 700°C., which contained little or no insoluble potassium. Perhaps later carbon dioxide determinations will explain the notable difference in the shape of Curves D and C. Curve B illustrates the heat behavior of an insoluble ash consisting largely of a double calcium-potassium phosphate. The similarity of this curve to the B curves of Charts 15 and 16 suggests that similar reactions took place. Later carbon dioxide determinations may confirm this.

Curve A, illustrating the losses under ignition of an insoluble ash derived from the reaction of monocalcium phosphate and calcium carbonate, seems to differ somewhat from the other three. It has a break in it as does Curve C, but it appeared at 700° instead of 600°C. Again, carbon dioxide determinations may explain the anomaly. The carbon dioxide results obtained on other insoluble ashes produced in the same manner as those illustrated on Chart 17 are given on Chart 18.

Curve A of Chart 18 shows the carbon dioxide content of the insoluble ash derived from the reaction between monocalcium phosphate and potassium carbonate under conditions favoring the production of a hydroxyapatite without inclusion of potassium in the molecule. It varied between four and two per cent according to the temperature. As the temperature rose the percentage of carbon dioxide in the insoluble ash decreased gradually and without any decided break in the curve, in contrast to the break in Curve A of Chart 17. The writer cannot at present explain this difference satisfactorily. Since no potassium entered the hydroxyapatite molecule, no calcium carbonate could have been liberated. This is corroborated by the absence of a break between 600° and 700°C. in Curve A. The carbon dioxide, therefore, could have come only from a carbonated apatite that releases the gas gradually from 500° to 800°C. and beyond. What was

said about carbonated apatites in connection with those produced by the action of sodium carbonate applies here. The only difference in the two reactions is the failure of the potassium to enter the hydroxyapatite and liberate some calcium carbonate. In the discussion in connection with Chart 9 it was noted that there was a shift in the experimental data even though there was no evidence of the presence of free calcium carbonate. A carbonated apatite would have the same effect until a temperature of 800°C. caused the volatilization of about 50 per cent of the carbon dioxide, and, very likely, a part of the water of constitution of hydroxyapatite. Curve F of Chart 9 now becomes logical.

Curve B is almost exactly like Curve B in Chart 16. The amount of soluble phosphorus pentoxide produced in the reaction of the potassium

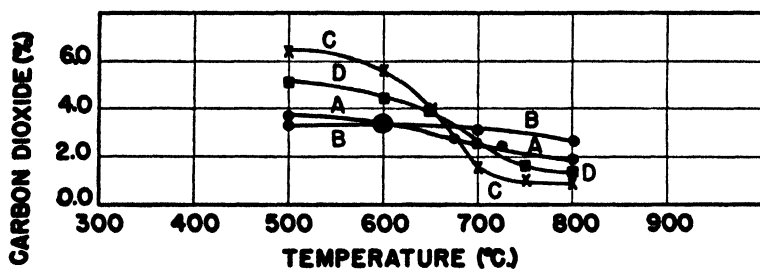


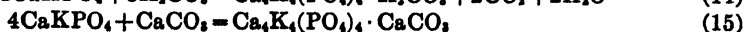
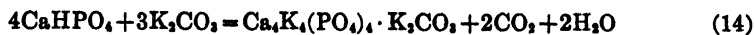
CHART 18.—CO<sub>2</sub> IN WATER-INSOLUBLE ASHES (%) DERIVED FROM REACTION OF CALCIUM PHOSPHATE AND POTASSIUM CARBONATE AT 500° AND 700°C., AT DIFFERENT TEMPERATURES

(Heating period at each point, 15 minutes)

A—Water-insoluble ash from  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  and  $\text{K}_2\text{CO}_3$  at 500°C. carbonate phosphate ratio over 5.0; B—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{K}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio = 0.512, crust well pulverized, conditions favorable for production of  $\text{CaKPO}_4$ , 250 mg. of  $\text{CaHPO}_4$  produced 22.0 mg. of water-soluble  $\text{P}_2\text{O}_5$ ; C—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{K}_2\text{CO}_3$  at 500°C., carbonate-phosphate ratio over 5.0, crust well pulverized, conditions favorable for production of a potassium-bearing hydroxyapatite; D—Water-insoluble ash from  $\text{CaHPO}_4$  and  $\text{K}_2\text{CO}_3$  at 700°C., carbonate-phosphate ratio over 5.0, crust well pulverized, previous work shows this water-insoluble ash contains no water-insoluble potassium.

carbonate on the monocalcium phosphate was also 22 mg. The lack of a break in the B curves on Charts 17 and 18 indicates the absence of calcium carbonate in spite of the production of soluble phosphates. Therefore, the existence of similar heat-resisting carbon dioxide in this insoluble ash should indicate that products analogous to those described in the previous sodium carbonate experiments were produced. Franck, Bredig, and Kanert<sup>13</sup> produced double calcium potassium phosphate and carbon dioxide containing rhenanites in 1938. The reactions are illustrated by Equations 14 and 15, analogous to Equations 11, 12, and 13.

<sup>13</sup> *Z. anorg. allgem. Chem.*, 237, 49 (1938).



Curves C and D represent the carbon dioxide data obtained on the insoluble ashes derived from the reaction of potassium carbonate on dicalcium phosphate at 500° and 700°C., respectively, with the same ratio of carbonate to phosphate (in excess of 5.0). Curve C on Chart 17 shows the calcium-carbonate break between 600° and 700°C. and Curve C on Chart 18 has also the same shape. These similarities indicate that the weight loss shown by Curve C on Chart 17 must have been due almost entirely to the volatilization of carbon dioxide, and very little, if any, to loss of water. The carbon dioxide content at 800°C. of about one per cent of the insoluble ashes represented by Curve C is to be especially noted. The insoluble potassium of the ash represented by Curve C was not determined but in the similar ash represented by Curve C of Chart 17 it amounted to 7.6 mg. These data all indicate that the insoluble ash in this case was a mixture of calcium carbonate and a potassium-containing hydroxyapatite that had been carbonated to some extent in a manner similar to analogous ashes in the sodium carbonate experiments.

Curve D represents an experiment similar to the experiment represented by Curve C, except that the temperature of reaction was 700°C. instead of 500°C. Data on Chart 17 show that no potassium is to be expected in this insoluble ash. The ash represented by Curve D started out with less carbon dioxide in it than that of C, and ended up with more at 800°C. The D curves on both Charts 17 and 18 have an appreciable but not exaggerated break between 600° and 700°C. This probably means that this insoluble ash had a remnant of calcium carbonate in it. It is also evident that it contained more of the carbonated hydroxyapatite (yielding carbon dioxide very slowly) than did the insoluble ash derived from the reaction at 500°C., due probably to the hotter temperature. A decrease of the calcium carbonate and an increase of the carbonated hydroxyapatite, which loses carbon dioxide gradually, could account for the crossing of Curves C and D of Chart 18 and for the change in shape of Curve C.

There remains for consideration the mechanism whereby the potassium-containing carbonated hydroxyapatite produced at 500°C. loses its potassium entirely at 700°C. in the presence of excess potassium carbonate, as shown on Chart 12. Curve D of Chart 18 suggests that when this happened the calcium carbonate that was produced at 500°C. likewise decreased. The writer believes that the calcium carbonate at 700°C. was able to knock the potassium from its rather unstable position in the framework of hydroxyapatite and allow calcium to take its place. In other words, there are two exchanges, the first at comparatively low temperatures and the other near 700°C. Carbonation of the hydroxyapatite by excess alkali carbonates seems to be an independent reaction.

The striking results obtained by this investigation of the reactions be-

tween calcium phosphates and alkali carbonates at ashing temperatures suggested that a similar study of the reactions of magnesium phosphates might be profitable. Such a study is now being conducted, and the results will appear in a future number of *This Journal*. The knowledge gained with the pure calcium and magnesium compounds will be applied later to the ashing of foods, or other agricultural products with an alkaline balance. It is expected that such products, if they contain appreciable quantities of calcium phosphates and also alkali salts that burn to carbonates on ashing, will react according to principles established in this investigation. Two phases of this study have been postponed for future considerations. They are (1) the effect of carbonation with carbon dioxide water (such as was proposed in the first paper of this series) on the properties of carbon dioxide combined in rhenanite or carbonated hydroxyapatite structure, and (2) modification of Equations 2-10 to account for the presence of carbon dioxide in insoluble ashes in the form of rhenanites and carbonated hydroxyapatites.

#### CONCLUSIONS

This investigation has shown that alkali carbonates can react with calcium phosphates, in both the wet and dry states, to form a number of different compounds, whose composition is governed principally by the carbonate/phosphate ratio and the temperature of ashing. The weight of the residue or total ash varies correspondingly to some degree. However, the composition of the ash, particularly of the insoluble ash, is more definitely influenced by these factors than is the weight of the total ash. Soluble phosphates are produced, and the amounts vary with the type of reaction and temperature. The greatest changes produced are those in the insoluble ash, which always contains sodium if sodium carbonate is one of the reactants. The insoluble ash may, or may not, contain potassium in analogous potassium carbonate reactions. If the carbonate/phosphate ratio is low (hardly more than that required for neutralization of acid calcium phosphates) the insoluble ash consists of insoluble double calcium-alkali phosphates, carbon dioxide-containing double phosphates (the so-called rhenanites) or mixtures of these. These carbonated double phosphates do not lose their carbon dioxide to any appreciable degree up to 800°C., which is considerably beyond ordinary ashing temperatures. Increase of the carbonate/phosphate ratio, as well as increase of temperature, tends progressively to force the reaction away from the double phosphate towards the hydroxyapatite equilibrium, with resulting decreases in the water-insoluble alkalis. Hydroxyapatites produced with sodium carbonate always contain sodium within the framework of the molecule, but potassium-containing hydroxyapatites are less stable. The hydroxyapatites produced at ashing temperatures always contain some carbon dioxide in a carbonated hydroxyapatite molecule, the exact position of which was

not determined in this investigation. The carbonated hydroxyapatites, when more or less isolated as insoluble ash, lose carbon dioxide gradually when heated at any temperature between 500° and 800°C. (the upper temperature limit of this investigation). When either sodium or potassium enters the hydroxyapatite molecule, an equivalent amount of calcium carbonate is set free and appears mixed with the hydroxyapatite in the insoluble ash. This calcium carbonate is sharply decomposed between 600° and 700°C. When a carbonated hydroxyapatite and calcium carbonate are heated together, the residues at increasing temperatures exhibit a decomposition curve that is less abrupt between 600° and 700°C. than is the case with calcium carbonate alone. The presence of carbon dioxide in the insoluble ash, whether combined in rhenanite structure, as carbonated hydroxyapatite, or as calcium carbonate, is the principal reason for the upward shift in the experimental curves away from the theoretical equilibrium. Excess alkali carbonate and high temperature (800°C.) in turn will decompose hydroxyapatites and cause more phosphorus to become water soluble. This accounts for the experimental curves crossing the horizontal line representing the pure hydroxyapatite equilibrium, and often even dropping a considerable distance below it at 800°C.

## ANALYSIS AND STABILITY OF ZINC PHOSPHIDE

By J. W. ELMORE and FRED J. ROTH (Bureau of Chemistry,\*  
California State Department of Agriculture, Sacramento, Calif.)

Owing to the shortage of strychnine and thallium sulfate since the beginning of the war, much of the poisoned-grain bait used for rodent control in California has been made with zinc phosphide. Although this compound has been used as a rodent poison for some time, it has not been used extensively, and little information is available with regard to its properties and stability under conditions of use or with regard to methods for the determination of the phosphide phosphorus content, particularly when present in small quantities in poisoned grain. The problem was complicated by the fact that pure zinc phosphide or material of known composition was not available to use as a standard of reference. For instance, zinc phosphide manufactured by one dealer bears a guarantee on the label of "Zinc phosphide 80%," but analysis indicated that this figure was a minimum and not the actual percentage of zinc phosphide present. Analysis of some of this material, hereinafter referred to as Sample C, gave total phosphorus 24.54 per cent and total zinc 74.21 per cent, corresponding to zinc phosphide 102.34 per cent and 97.90 per cent, respectively.

A method of analysis that would distinguish phosphide phosphorus

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\* Alvin J. Cox, Chief.

from other forms was desired. Preliminary attempts to determine the phosphide phosphorus by direct oxidation with hypochlorite or bromide-bromate solution proved unsuccessful. Some method based on the evolution of phosphine was considered feasible and should have an advantage in that it could be applied to zinc phosphide-poisoned grain as well as to zinc phosphide itself. Therefore, the problem resolved itself into a search for conditions that would give maximum conversion of zinc phosphide into phosphine. References indicated that dilute acids would liberate phosphine from zinc phosphide according to the reaction,  $\text{Zn}_3\text{P}_2 + 6\text{HCl} = 3\text{ZnCl}_2 + 2\text{PH}_3$ .

#### EXPERIMENTAL

It was thought that once the evolved phosphine was caught and oxidized to phosphate, it could be determined by the usual volumetric procedure used in fertilizer analysis. Preliminary attempts were made to develop this idea by using different strengths of hydrochloric acid, different forms of apparatus, different oxidizing agents, and different periods of time and temperatures.

*Evolution and Oxidation of Phosphine.*—Different hydrochloric acid strengths were experimented with, and a 1:3 strength was found to be satisfactory and selected for use. The evolved phosphine was carried in a current of gas into scrubbers for oxidation. Since some of the hydrides of phosphorus will spontaneously ignite in air it was at first thought that special precautions would be necessary to keep oxygen from the phosphine after it was liberated in the reaction flask. For this reason, in the early experimental work an atmosphere of natural gas (city gas) was used. However, such a precaution was found to be unnecessary. No appreciable oxidation of phosphine by air occurs since the same results were obtained with air as with gas. Initial attempts to oxidize phosphine to phosphate with hypochlorite were unsuccessful, as recoveries by the procedure varied from 50 to 70 per cent. When the writers discovered that hypochlorite solution was not giving complete oxidation, they followed the suggestion of H. Reckleben,<sup>1</sup> and tried potassium permanganate solution as an oxidizing agent the reaction being represented by the equation,  $5\text{PH}_3 + 8\text{KMnO}_4 + 12\text{H}_2\text{SO}_4 = 5\text{H}_3\text{PO}_4 + 8\text{MnSO}_4 + 4\text{K}_2\text{SO}_4 + 12\text{H}_2\text{O}$ . This gave complete conversion to phosphate, and under the conditions of the experiment it was found that 95 to 99 per cent of the liberated phosphine is oxidized to phosphate in the first absorption flask at room temperature, the remainder being completely absorbed in the second flask, as proved by trials of a third flask. Straight tube bubblers were used. Ordinary fritted glass bubblers are unsatisfactory since they become clogged from precipitation of manganese dioxide. After the oxidation reaction is complete, reduction of all manganese compounds is necessary to clear the solution. This is effected by passing sulfur dioxide into the solution. The

<sup>1</sup> *Z. anal. Chem.*, 54, 308 (1915); *Chem. Abstracts*, 9, 2746.

phosphate was finally determined by titration of ammonium phosphomolybdate according to the A.O.A.C. volumetric procedure.

*Temperature and Time.*—A factor giving some difficulty was the optimum temperature of the reaction flask. It was found that although air was drawn through the apparatus at room temperature until no more phosphine was evolved, the total amount of phosphorus obtained was not so much as was obtained by carrying out the reaction at 48°–50°C. The following table shows the temperature and time results of the analysis of zinc phosphide samples from the same manufacturer.

TABLE 1.—Zinc phosphide calculated from phosphine (per cent)

SAMPLE	C	D
<i>Hours of treatment</i>	Evolved at room temperature	
4	88.53	90.06
7	89.32	92.55
10	90.14	92.55
13	90.14	
	Evolved at 48°–50°C.	
$\frac{1}{2}$	91.44	
1	92.47	
$1\frac{1}{2}$	92.41	
2	92.25	
4	92.30	

It appears that at 50°C. no further phosphine is evolved after the first hour, and that therefore this length of time is sufficient to remove all available phosphine.

*Accuracy.*—In order to determine whether the reagents used to absorb and oxidize the phosphine caused any interference in the volumetric determination of phosphate, check analyses were made with known amounts of phosphate added to a permanganate solution, treated with sulfur dioxide, and analyzed. The variation from the theoretical was found to be less than plus or minus 0.2 per cent of the amount taken. Variations in results of analysis of zinc phosphide, principally due to uncertainty of the titration end point, are from 0.25 to 0.50 per cent of the amount present; however, these influence only the third decimal with respect to zinc phosphide poisoned grain.

*Sample C.*—A method of analysis supplied by Merck and Co., based on a direct reaction between zinc phosphide and silver nitrate, gave results for Sample C well above the guarantee on this material, but lower than obtained by the evolution method presented here. From results on this same sample by the latter method, phosphine phosphorus was calculated as 22.20 per cent. The phosphorus not evolved as phosphine was 2.34 per cent; of this 0.33 per cent was found as phosphate and the re-



mainder in combination with zinc as a black insoluble residue. The ratio of phosphorus to zinc in this residue was greater than that corresponding to the formula  $\text{Zn}_3\text{P}_2$ . Some of this material was fed to rats in doses several times greater than the lethal dose of zinc phosphide with no apparent effects, which indicates that all the toxic phosphorus is obtained in the analytical procedure.

#### METHOD FOR DETERMINATION OF PHOSPHIDE PHOSPHORUS IN ZINC PHOSPHIDE AND ZINC PHOSPHIDE-POISONED GRAINS

In view of the experimental results the following procedure is in use by the Bureau of Chemistry for the evaluation of zinc phosphide and zinc phosphide-poisoned grains.

##### REAGENTS

*Potassium permanganate solution.*—1.5%

*Hydrochloric acid.*—(1+3)

*Sulfur dioxide*

*Ammonium nitrate solution.*—10%

*Molybdate solution.*—See *Methods of Analysis*, A.O.A.C., 1940, p. 21, par. 7(a).

##### APPARATUS

The reaction flask is connected with two straight-tube, gas, wash bottles, B, not fritted glass bubblers, of approximately 300 ml. capacity (tall form), as shown in Figure 1.

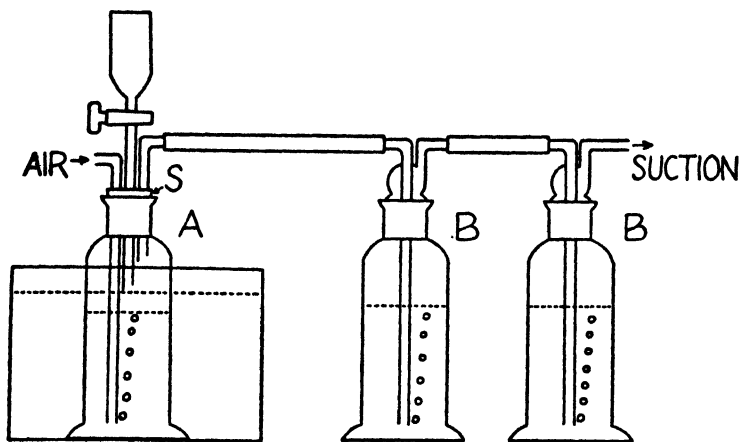


FIG. 1.—REACTION FLASK AND ABSORPTION TRAIN FOR EVOLUTION AND ABSORPTION OF  $\text{PH}_3$  IN THE ANALYSIS OF  $\text{Zn}_3\text{P}_2$ .

The reaction flask, A, having a capacity of about 150 ml., is fitted with a stopper, S, carrying a dropping funnel and outlet tube. The air inlet tube extends to the bottom of the reaction flask, which is immersed in a water bath heated to  $48^\circ\text{--}50^\circ\text{C}$ . The contents of the cylinder should be kept well stirred by the air flow during aspiration.

##### PROCEDURE

Place 200 ml. of the permanganate solution in each of the gas wash bottles, B.

Weigh 0.25 gram of the  $\text{Zn}_3\text{P}_2$  sample or 25 grams of zinc phosphide-poisoned grain and place in A. (Zinc phosphide may be conveniently weighed out in a 2 grain pharmacist's gelatin capsule.) Replace the stopper, S, start the suction, and add 75 ml. of the  $\text{HCl}$  to A through the funnel. With the reaction flask heated to  $48^\circ\text{--}50^\circ\text{C}$ ., draw air through the apparatus, about six bubbles per second, for one hour or longer if the rate of aspiration is slower. Disconnect and pass  $\text{SO}_2$  into the cylinders containing the permanganate solution until clear. Transfer the contents of each cylinder into a separate 500 ml. volumetric flask. Cool, and make to volume. Take 100 ml. from each volumetric flask and boil 5 minutes to remove  $\text{SO}_2$ . Cool, add 10 ml. of the  $\text{NH}_4\text{NO}_3$  solution and 35 ml. of the molybdate solution. Determine the  $\text{P}_2\text{O}_5$  content by the volumetric A.O.A.C. procedure (*Methods of Analysis*, A.O.A.C., 1940, p. 22, par. 12), beginning with the words "Place solution in a shaking or stirring apparatus . . ." Calculate zinc phosphide, using the relationship,  $\text{P}_2\text{O}_5 \times 1.818 = \text{Zn}_3\text{P}_2$ .

### NOTES ON STABILITY OF ZINC PHOSPHIDE

The stability of zinc phosphide on poisoned grain is a matter of considerable interest to those engaged in rodent control work. Aluminum phosphide and calcium phosphide are known to react with water to give vari-

TABLE 2.—*Analysis of exposed zinc phosphide-poisoned grain*

TIME OF EXPOSURE	ZINC PHOSPHIDE FOUND
days	per cent
None	0.56
8	0.51
14	0.44
21	0.40
29	0.40

ous hydrides of phosphorus.<sup>2</sup> Zinc phosphide is reported to give off phosphorus vapor in contact with the air,<sup>3</sup> and the odor of phosphorus can be detected about the material. In view of these observations it was anticipated that zinc phosphide would show only a limited stability. To test this matter, trays of zinc phosphide-poisoned grain were exposed to the weather on a roof and analyzed periodically. The results are shown in Table 2.

Rain fell on the samples on the 16th and 20th days, but as they were also subjected to considerable wind and dust, it was thought that the diminishing phosphide content might have been due to mechanical factors rather than decomposition.

In order to determine whether zinc phosphide-treated grains deteriorate under conditions of ordinary storage, a burlap bag of this material was kept in the laboratory storeroom and analyses were made at 30-day intervals up to 180 days. The zinc phosphide content remained constant throughout the entire period.

<sup>2</sup> Arthur J. Hale, "Modern Chemistry, Pure and Applied," vol. 1, p. 215.

<sup>3</sup> "U. S. Dispensary," 22nd ed., p. 1648.

In another case the mechanical action of heavy rains on the poisoned grain as scattered in a field was studied. It was found that a prepared bait was reduced from an original analysis of 0.21 per cent of zinc phosphide to 0.14 per cent after 2 days' exposure to heavy rains.

Another sample, the original analysis of which was 0.50 per cent of zinc phosphide, was exposed to outside conditions but protected from rains. After one month the analysis showed 0.47 per cent, and after 9 months' exposure, 0.40 per cent of zinc phosphide.

In general, it may be concluded that there is practically no chemical change in zinc phosphide on poisoned grains under conditions of storage but in field use some deterioration of baits may be expected, the rate depending chiefly on mechanical factors of rains and winds.

## WATER SOLUTIONS OF SUPERPHOSPHATE AS A LOW-FLUORINE SOURCE OF PHOSPHORUS

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The utilization of superphosphate by the farmer for the preparation of low-fluorine phosphate for animal feeding was recently proposed in the Union of South Africa (5, 7, 8). One of the means recommended for accomplishing this end is to introduce the phosphate into the animals' drinking water. In this procedure the phosphate solution is prepared by agitating 50 pounds of either ordinary or double superphosphate\* with 40 gallons of water, allowing the mixture to settle, and decanting the clear solution, which is then diluted to the proper concentration (0.4 gram per liter) in the watering trough. Tests (7) indicated that the fluorine concentration of the dilute solution was approximately 4 parts per million. The use of solutions of double superphosphate in animal feeding has also been studied to some extent in the United States† since 1941, but as yet the results have not been published. Although this method of providing a low-fluorine phosphate supplement has considerable promise in areas where the supply of drinking water can be controlled, the published data do not give the results of a systematic study that would indicate the best conditions for preparing the solution from superphosphate. The results of such a study are reported in this paper.

\* The term *ordinary superphosphate* refers to the fertilizer material made by treating phosphate rock with  $\text{H}_2\text{SO}_4$ . It usually contains 18–22% of  $\text{P}_2\text{O}_5$  and ca. equal parts of monocalcium phosphate and calcium sulfate. The term *double superphosphate* refers to the fertilizer material made by treating phosphate rock with  $\text{H}_3\text{PO}_4$ . It contains 44–51% of  $\text{P}_2\text{O}_5$  and is frequently called *triple superphosphate* or *treble superphosphate* in commerce.

† N. R. Ellis, Bureau of Animal Industry, Beltsville, Md., private communication.

## MATERIALS

The fluorine content of ordinary superphosphate produced in this country ranges from 1.2 to 2.0 per cent, whereas double superphosphate carries 1.5 to 3.9 per cent (Table 1). The superphosphates used in this study were cured materials representing a wide sampling of domestic production. The necessary descriptive data for the superphosphates, being pertinent to the results there given, are assembled in a later table (Table 4). Materials numbered in the P-series and No. 2216 were produced in the winter and spring of 1943; the others, samples used by Tremearne and Jacob (9), were produced in 1939 and 1940.

## PREPARATION OF SOLUTION OF SUPERPHOSPHATE

Several factors—fineness of sample, agitation, and superphosphate-water ratio—that might be expected to influence the quantities of phosphate and fluorine brought into solution were investigated to determine the optimal conditions for a standard procedure for the preparation of test solutions.

*Standard procedure.*—To 7 grams of ordinary superphosphate, or 5 grams of double superphosphate, in a 200 ml. Erlenmeyer flask was added 100 ml. of distilled water, and the mixture was agitated for 30 seconds by swirling. The agitation was repeated at the end of 5 minutes and 2 hours, respectively. The mixture was allowed to stand at room temperature overnight (18–20 hours), whereupon aliquots of the clear solution, carefully drawn to avoid disturbing the sediment, were analyzed for fluorine and phosphoric oxide. The mixtures were prepared in duplicate from the unground superphosphates.

The agreement between single determinations on duplicate solutions was perhaps better than might be expected. The differences of 70 pairs of phosphoric oxide titrations ranged from 0.0 to 0.6 mg. and averaged 0.17 mg., which corresponds to 0.07 gram per liter for ordinary superphosphates and, because of the smaller aliquot, to 0.17 gram per liter for double superphosphates. The differences of the 70 pairs of fluorine results ranged from 0.000 to 0.023 gram per liter with an average of 0.005 gram per liter.

The reproducibility of the data in the tables is indicated by the agreement between results obtained on pairs of mixtures that were replicated at different times. Thus, the values obtained on ordinary superphosphate P2248 were 11.1, 11.3, and 11.4 grams of phosphoric oxide per liter, and the corresponding figures for fluorine were 0.140, 0.147, and 0.134 gram per liter. Comparable results for double superphosphate 1900 were: phosphoric oxide 15.7, 15.9, and 16.1; fluorine 0.282, 0.275, and 0.275.

*Fineness of sample.*—The superphosphates varied widely with respect to fineness. In some instances the material was uniformly about 20 mesh; in others the sample was a mixture of fine material with lumps as large as a pea. Results on one of the least uniform materials (P2068) indicated

TABLE 1.—Phosphoric oxide and fluorine contents of domestic superphosphate<sup>a</sup>

ITEM NO.	SUPERPHOSPHATE		SAMPLES INCLUDED IN AVERAGE	TOTAL P <sub>2</sub> O <sub>5</sub>		F	
	MADE FROM—	CHARACTER OF MATERIAL OR OF INGREDIENT ACID		RANGE	AVERAGE	RANGE	AVERAGE
Ordinary Superphosphate							
1	Florida land pebble	Den material	9	19.1 to 22.5	20.6	1.5 to 2.0	1.7
2	Florida land pebble	Cured material	22	17.9 to 22.2	20.3	1.4 to 1.9	1.7
3	Tennessee brown rock	Den material	9	19.2 to 22.2	20.0	1.2 to 1.7	1.4
4	Tennessee brown rock	Cured material	7	18.9 to 21.9	20.7	1.3 to 1.7	1.5
5	Idaho and Montana rock	Cured material	4	20.0 to 21.5	20.5	1.3 to 1.6	1.4
Double Superphosphate							
6	Florida land pebble	Wet-process acid	7	46.2 to 49.7	48.7	1.7 to 2.7	2.0
7	Florida land pebble	Furnace-process <sup>b</sup> acid	2	50.3 to 50.6	50.5	1.7	1.7
8	Tennessee brown rock	Wet-process acid	8	43.9 to 48.7	46.5	2.0 to 3.9	3.1
9	Tennessee brown rock	Furnace-process <sup>b</sup> acid	4	47.5 to 50.9	49.2	1.5 to 1.7	1.6
10	Idaho and Montana rock	Wet-process acid	8	45.1 to 47.6	46.7	1.8 to 3.0	2.1

<sup>a</sup> Compiled from data of Jacob et al. (4) and the results reported in this paper that fall in Items 2, 4, and 5.<sup>b</sup> Electric furnace.

that grinding the lumps to pass the 20-mesh sieve had little effect on the composition of the solution. For example, results obtained by the standard procedure on ground and unground sample, respectively, were: dissolved phosphoric oxide 9.71 and 9.83 grams per liter, dissolved fluorine 0.075 and 0.070 gram per liter. With the use of 3 grams of superphosphate closer agreement was obtained: phosphoric oxide 4.30 and 4.31, fluorine 0.061 and 0.061.

*Agitation.*—The first question that arises in this connection is whether or not the third stirring specified in the standard procedure is worthwhile. The results in Table 2 indicate that in the case of ordinary superphosphate the third stirring brings into solution only 2–4 per cent more phos-

TABLE 2.—*Effect of agitation on the composition of the solution*

SUPERPHOSPHATE NO.	CONSTITUENT DISSOLVED IN DUPLICATE RUNS WITH TWO AGITATIONS		CONSTITUENT DISSOLVED IN DUPLICATE RUNS WITH THREE AGITATIONS	
	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE
	gram/liter	gram/liter	gram/liter	gram/liter
Phosphoric oxide ( $P_2O_5$ )				
P2115	10.32	0.04	10.54	0.08
P2126	11.45	0.30	11.64	0.20
P2077	12.00	0.80	12.42	0.60
Fluorine (F)				
P2115	0.242	0.014	0.279	0.008
P2126	0.131	0.002	0.136	0.000
P2077	0.103	0.002	0.108	0.011

phoric oxide and 4–15 per cent more fluorine with a resultant tendency toward an increase in the F- $P_2O_5$  ratio of the solution. On the other hand, the agreement of the phosphoric oxide results on duplicate solutions was somewhat improved by the third agitation, which justifies its inclusion in the standard procedure.

Experiments were also conducted to determine the effect of allowing the mixture to stand several days with occasional agitation. After aliquots had been drawn from the mixture prepared in accordance with the standard procedure the residual mixture was agitated once each day for 4 days and then analyzed in the usual way on the fifth day. The data (Table 3) show increases in dissolved phosphoric oxide up to 0.3 gram per liter, which are scarcely outside the indicated limits of reproducibility, whereas the alterations in the amounts of dissolved fluorine are about equally divided between positive and negative differences. Accordingly, little would seem to be gained by aging the mixture beyond the time (20 hours or less) required for it to settle to a clear solution.

*Superphosphate-water ratio.*—The dependence of the composition of the

solution on the ratio of superphosphate to water used in preparing the mixture, as reflected by the dissolved phosphoric oxide and fluorine and by the ratio  $F:P_2O_5$  in solution, is shown in Figures 1 and 2. The curves for ordinary superphosphate (Figure 1) become nearly horizontal at about 6 grams per 100 ml. of water, and further increase in the proportion of superphosphate is not accompanied by a significant reduction in the

TABLE 3.—*Effect of standing time on composition of solution*

SUPERPHOSPHATE		SUPERPHOSPHATE-WATER MIXTURE			
NO.	MADE FROM—	TIME OF STANDING	DISSOLVED P <sub>2</sub> O <sub>5</sub>	DISSOLVED F	F:P <sub>2</sub> O <sub>5</sub>
		days	gram/liter	gram/liter	
Ordinary Superphosphate					
P2248	Florida land pebble	1	11.3	0.140	0.0124
		5	11.3	.177	0.0157
P2126	Florida land pebble	1	11.6	.136	0.0117
		5	11.7	.102	0.0087
1896	Tennessee brown rock	1	10.7	.078	0.0073
		5	11.0	.117	0.0106
P2131	Idaho rock	1	12.1	.250	0.0207
		5	12.4	.281	0.0227
Double Superphosphate					
1880 <sup>a</sup>	Florida land pebble	1	13.1	0.098	0.0075
		4	13.3	.088	0.0066
2216 <sup>a</sup>	Tennessee brown rock	1	11.8	.055	0.0047
		4	11.9	.048	0.0040
1906	Idaho rock	1	18.1	.063	0.0035
		4	18.2	.069	0.0038

\* The mixture contained 3 grams of superphosphate to 100 ml. of water.

amount of dissolved fluorine relative to the phosphate. The curves for double superphosphate (Figure 2) have the same general form, but, owing to the higher phosphate content of the superphosphate, they level off around 5 grams per 100 ml. of water. The small slope of the curves at the higher ratios means that higher ratios than those mentioned above and adopted in the standard procedure could be used without serious reduction in the percentage of the phosphoric oxide dissolved. The upper limit of the concentration of phosphoric oxide is probably near that of the solution in univariant equilibrium with mono- and dicalcium phosphate in the

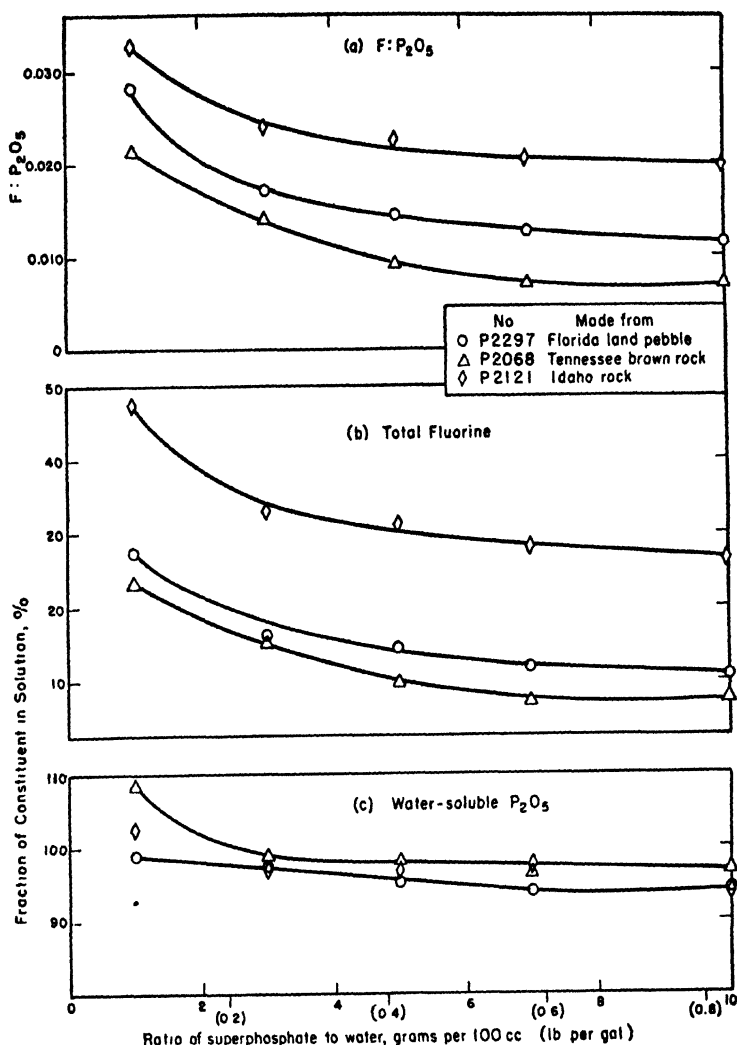


FIG. 1.—DEPENDENCE OF COMPOSITION OF SOLUTIONS OF ORDINARY SUPERPHOSPHATE ON THE SUPERPHOSPHATE-WATER RATIO

system  $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ , which at  $25^\circ\text{C}$ . contains 313.7 grams per liter (2). The attainment of the maximal concentration of dissolved phosphoric oxide in superphosphate-water mixtures would necessitate the conversion of considerable water-soluble monocalcium phosphate to water-insoluble dicalcium phosphate and would also require an impractically large proportion of superphosphate.



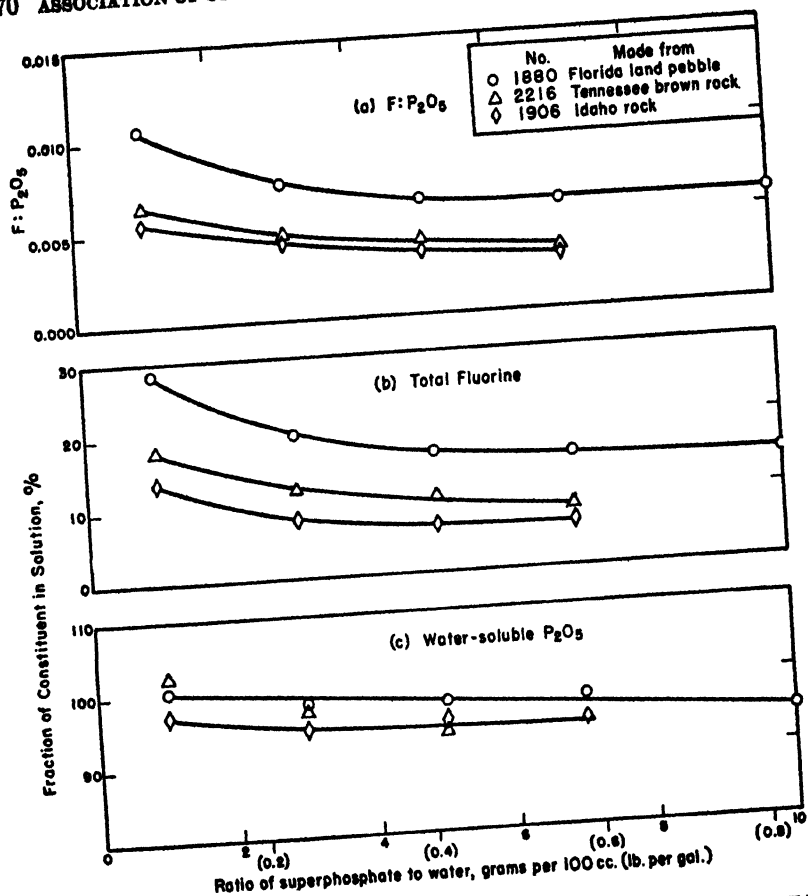


FIG. 2.—DEPENDENCE OF COMPOSITION OF SOLUTIONS OF DOUBLE SUPERPHOSPHATE ON THE SUPERPHOSPHATE-WATER RATIO.

#### SOLUTIONS FROM DIFFERENT SUPERPHOSPHATES

The composition of solutions prepared by the standard procedure from 18 superphosphates is given in Table 4, which also includes relevant data relating to the composition of the superphosphates. Of the water-soluble phosphoric oxide found by the official method (column 4) 90–100 per cent and more (average 95.4 per cent) was dissolved in the superphosphate-water mixtures (column 9) of both ordinary and double superphosphate, whereas only 2–28 per cent of the total fluorine was contained in the clear solution (column 11). Thus, in all cases the dissolved phosphate was in effect partially defluorinated as a result of disproportionate partition of the fluorine between solution and insoluble residue. The extent of defluorination, which may be more readily appreciated by comparing the  $F:P_2O_5$  ratio (columns 7 and 12), varied from 68 to 98 per cent. The

TABLE 4.—Composition of typical superphosphates and their water solutions<sup>a</sup>

1	2	3	4	5	6	7	8	9	10	11	12
SUPERPHOSPHATE											
NO.	MADE FROM—	TOTAL P <sub>2</sub> O <sub>5</sub>	WATER- SOLUBLE P <sub>2</sub> O <sub>5</sub> <sup>b</sup>	CITRATE- INSOLUBLE P <sub>2</sub> O <sub>5</sub> <sup>b</sup>	F	F:P <sub>2</sub> O <sub>5</sub>	SUPERPHOSPHATE-WATER MIXTURE				
		per cent	per cent	per cent	per cent	gram/liter	DISSOLVED P <sub>2</sub> O <sub>5</sub>		DISSOLVED F		F:P <sub>2</sub> O <sub>5</sub>
							per cent of Water-soluble	gram/liter	per cent of total		
Ordinary Superphosphate											
P2115	Florida land pebble	19.2	16.6	1.01	1.64	0.0854	10.5	90.5	0.279	24.3	0.0267
P2035		20.0	17.6	0.11	1.72	.0860	11.7	95.0	.093	7.7	.0079
P2248		20.5	16.9	1.40	1.75	.0854	11.3	95.5	.140	11.4	.0124
P2126		20.6	17.4	0.10	1.75	.0850	11.6	95.3	.136	11.1	.0117
P2297		20.7	18.5	0.98	1.89	.0913	12.2	94.2	.155	11.7	.0127
P2134		20.8	17.9	0.44	1.82	.0875	12.2	97.4	.073	5.7	.0060
P2077		21.0	18.2	0.77	1.83	.0871	12.4	97.4	.108	8.4	.0087
P2184		22.2	18.7	0.12	1.85	.0834	12.5	95.5	.098	7.6	.0078
2007	Tennessee brown rock	19.2	14.1	0.07	1.42	.0740	9.35	94.7	.096	9.7	.0103
1983		19.8	15.7	0.10	1.46	.0738	10.3	93.8	.018	1.8	.0017
1896		21.4	15.2	0.19	1.67	.0780	10.7	100.6	.078	6.7	.0073
P2068		21.9	14.6	1.10	1.32	.0603	9.83	96.2	.070	7.6	.0071
P2131	Idaho rock	20.4	17.9	0.36	1.26	.0618	12.1	96.6	.250	28.3	.0207
Double Superphosphate											
1878	Florida land pebble	48.8	42.9	0.22	2.68	0.0549	20.6	96.0	0.328	24.5	0.0159
1880		49.5	44.1	0.16	1.65	.0333	21.6	98.0	.136	16.5	.0063
1900	Tennessee brown rock	47.0	34.0	0.44	3.26	.0694	15.9	93.6	.277	17.0	.0174
2216		47.7	40.4	2.41	1.50	.0315	18.9	93.6	.078	10.4	.0041
1906	Idaho rock	46.9	38.2	3.08	1.88	.0402	18.1	94.8	.063	6.7	.0035

<sup>a</sup> Prepared by the described standard procedure.<sup>b</sup> Determined by the official method for available phosphates in fertilizers (1, p. 23).

amounts of fluorine in solution (column 10) varied in an irregular manner and ranged from 0.018 to 0.279 gram per liter. The phosphoric oxide concentration (column 8) depends on the content of water-soluble phosphate (column 4) in the superphosphate and amounted to 9.3–12.5 grams per liter for ordinary superphosphate and to 15.9–21.6 grams per liter for double superphosphate.

#### DEFLUORINATION BY ADDITION OF REAGENTS

Experiments were conducted with a view toward finding readily obtainable reagents that would depress the fluorine concentration of the solution without serious lowering of its phosphate content. To this end a series of tests was made in accordance with the standard procedure, in which sodium chloride, sodium silicate, bone ash, calcium carbonate (C.P. grade and limestone), dolomite, and agricultural burned lime, respectively, were added to the superphosphate-water mixture immediately before the second agitation. The results are assembled in Table 5. Only sodium silicate, the calcium carbonates and the agricultural burned lime showed a marked lowering of the fluorine concentration, and of these the calcium carbonates caused the least reduction of the phosphate concentration. Thus, the addition of 0.5 gram of limestone to an ordinary superphosphate-water mixture reduced the dissolved fluorine from 0.140 to 0.054 gram per liter (Items 1 and 11) and the phosphoric oxide concentration from 11.3 to 10.9 grams per liter. The effect on a solution of double superphosphate (Item 19) was less marked.

#### APPLICATIONS TO ANIMAL FEEDING

The foregoing results show that phosphate solutions carrying 9–12 grams of phosphoric oxide per liter with 0.02–0.28 gram of fluorine per liter can be readily prepared from ordinary superphosphate with the use of 7 grams to 100 ml. of water, or a little more than 0.5 pound per gallon. On dilution to a suitable concentration for the animals' drinking water, say 0.4 gram of phosphoric oxide per liter (7), the fluorine concentration would be reduced to 1–11 parts per million. Similarly, with the use of double superphosphate at the rate of 5 grams per 100 ml. of water, or somewhat less than 0.5 pound per gallon, the solution would contain 16–22 grams of phosphoric oxide per liter with 0.06–0.33 gram of fluorine per liter, which on dilution as before would yield a fluorine concentration of 1.5 to 6 parts per million. The upper limits indicated for the fluorine concentrations can be substantially reduced by the addition of a relatively small quantity of ground limestone to the superphosphate-water mixture (Table 5). Furthermore, the phosphoric oxide concentration can be roughly doubled by increasing the proportion of superphosphate in the initial mixture to approximately 1 pound per gallon, the amount recommended by du Toit et al (7), without significant alteration of the amount of fluorine relative to the phosphate in solution.

TABLE 5.—*Effect of added reagents on the composition of the solution*

ITEM NO.	MATERIAL ADDED	DISSOLVED P <sub>2</sub> O <sub>5</sub>	DISSOLVED F	F:P <sub>2</sub> O <sub>5</sub>
		gram/liter	gram/liter	
Ordinary Superphosphate <sup>a</sup>				
1	None	11.3	0.140	0.0124
2 <sup>b</sup>	None	11.5	0.127	0.0110
3	NaCl, 0.5 grams	11.2	0.150	0.0133
4	1.0	11.2	0.136	0.0122
5 <sup>c</sup>	1.0	11.5	0.111	0.0097
6	1.5	11.2	0.145	0.0130
7	Sodium silicate, <sup>d</sup> 0.2 cc.	8.7	0.045	0.0051
8	Bone ash, 0.5 gram	11.0	0.123	0.0112
	Calcium carbonate			
9	Precipitated, C.P., 0.5 gram	10.6	0.030	0.0028
10	Limestone, <sup>e</sup> 100+150 mesh, 0.5 gram	11.0	0.061	0.0054
11	Limestone, <sup>e</sup> 100 mesh, 0.5 gram	10.9	0.054	0.0049
12	Dolomite, <sup>f</sup> 100 mesh, 0.5 gram	11.3	0.122	0.0108
Double Superphosphate <sup>a</sup>				
13	None	15.9	0.277	0.0174
14	NaCl, 1.0 gram	15.7	0.251	0.0160
15	Sodium silicate, <sup>d</sup> 0.2 cc.	12.6	0.049	0.0039
16	Bone ash, 0.5 gram	16.1	0.236	0.0147
	Calcium carbonate			
17	Precipitated, C.P., 0.5 gram	15.2	0.032	0.0021
18	Limestone, <sup>e</sup> 100+150 mesh, 0.5 gram	15.2	0.195	0.0128
19	Limestone, <sup>e</sup> 100 mesh, 0.5 gram	15.1	0.207	0.0137
20	Dolomite, <sup>f</sup> 100 mesh, 0.5 gram	15.7	0.245	0.0156
21	Agricultural burned lime, <sup>h</sup> 0.34 gram	12.1	0.023	0.0019

<sup>a</sup> Florida land-pebble superphosphate P2248 (Table 4) was used.<sup>b</sup> The solution of Item 1 that had stood for 5 days with one shaking each day.<sup>c</sup> The solution of Item 4 that had stood for 5 days with one shaking each day.<sup>d</sup> Material was sold as egg preservative.<sup>e</sup> Analysis showed 98.4% CaCO<sub>3</sub> by titration.<sup>f</sup> The producers' analysis showed 55.0% CaCO<sub>3</sub> and 44.5% MgCO<sub>3</sub>.<sup>g</sup> Florida land-pebble superphosphate 1900 (Table 4) was used.<sup>h</sup> Analysis showed CaO 85.2% by titration and ignition loss 14.2%.

Calcium was not determined in the solutions of superphosphate, but on the basis of data, recorded elsewhere (3), for the contents of monocalcium phosphate and free phosphoric acid in superphosphate the calcium oxide concentration of the solutions prepared by the standard procedure would be expected to lie in the range 5–10 grams per liter.

Ordinary superphosphate carries calcium sulfate to the extent of approximately 30 per cent sulfur trioxide, and up to 6.5 per cent sulfur trioxide has been reported in double superphosphate (3). The sulfate content of solutions prepared from ordinary superphosphate by the standard procedure was approximately 1 gram of sulfur trioxide per liter and it was slightly higher when the proportion of superphosphate was doubled. For example, solutions prepared with the use of 7 and 14 grams of superphosphate P2248 carried 1.14 and 1.29 grams of sulfur trioxide per liter, respectively.

TABLE 6.—*Arsenic content of domestic superphosphates*<sup>a</sup>

ITEM NO.	SOURCE OF H <sub>2</sub> SO <sub>4</sub>	TYPE OR SOURCE OF PHOSPHATE ROCK <sup>b</sup>	SAMPLES INCLUDED IN AVERAGE	P <sub>2</sub> O <sub>5</sub>		As	
				RANGE	AVERAGE	RANGE	AVERAGE
Ordinary Superphosphate							
1	Sulfur	P B	6	19.8 to 21.9	20.9	4.1 to 14.3	7.6
2	Copper smelter	P B	4	20.3 to 21.4	20.7	6.0 to 11.3	8.7
3	Zinc smelter	B	2	20.0 to 21.2	20.6	2.2 to 35.7	19.0
4	Spanish pyrites and sulfur	P	3	20.8 to 21.0	20.9	494 to 1199	930
Double Superphosphate							
5	Sulfur	P	2	48.8 to 49.5	49.2	10.6 to 11.6	11.1
6 <sup>c</sup>	—	B P	3	47.5 to 50.7	49.6	11.1 to 22.9	16.1
7	Copper smelter	B	2	44.1 to 47.0	45.6	20.8 to 34.3	27.6
8	Zinc smelter	M	1	—	45.7	—	64.9
9 <sup>d</sup>	—	B	2	48.4 to 50.3	49.4	100 to 119	110
10	Iron sulfide concentrate	I	1	—	47.3	—	405

<sup>a</sup> Compiled from data of Tremearne and Jacob (9).<sup>b</sup> Abbreviations used are: B Tennessee brown rock, I Idaho rock, M Montana rock, P Florida land pebble.<sup>c</sup> Phosphoric acid was prepared by electric-furnace process.<sup>d</sup> Phosphoric acid was prepared by blast-furnace process.

All grades of superphosphate contain arsenic (Table 6). Materials prepared with the use of sulfuric acid obtained by burning sulfur or with the use of phosphoric acid produced by the electric-furnace process comprise a low-arsenic group (Items 1, 5 and 6), for which the arsenic content ranges from 4 to 23 parts per million. If all the arsenic should be dissolved, its concentration in the dilute solutions discussed above would scarcely exceed 1 part per million. A matter of interest in this connection is that amounts of arsenic ranging from 0.6 to 2.2 parts per million have been reported in bone meal (9). Lead is an undesirable element that might be suspected in superphosphate, but apparently no systematic search has been made for it. Up to 4 parts of selenium per million have been reported in superphosphate (6). Vanadium and chromium are known to occur to the extent of several tenths per cent of the trioxides in certain phosphate rocks of the western United States.

Solutions of superphosphate can be safely prepared in, and administered from, vessels of iron, wood, masonry, or concrete. The action of such solutions on galvanized vessels has not been studied, but owing to the toxicity of soluble zinc to animals this type of container should be avoided.

Factors that limit the application of water solutions of superphosphate to animal feeding—control of drinking-water supply, hardness of the water, attitude of farmer, variations in phosphorus deficiency from district to district, and the economic aspect of necessary installations—are discussed by van der Merwe (5) and need not be reviewed here. It should be pointed out, however, that superphosphate for this use would necessarily be evaluated on the basis of water-soluble phosphoric oxide rather than the available phosphoric oxide customary in the fertilizer trade in this country. Nevertheless, the phosphate (13–36 per cent of the phosphoric oxide in the superphosphate) contained in the fluorine-rich insoluble residue, and thereby lost for feeding purposes, may be utilized by application to the soil as a fertilizer.

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## NOTE

### Loss in Weight of Platinum Dishes\*

A record of the loss in weight of platinum dishes used in this laboratory in routine fusions and evaporations in connection with the analysis of jams, jellies, fruit juices, sugar and sugar products, inorganic salts, and water samples has been kept for approximately 30 years.

All analytical work was done according to the A.O.A.C. methods of analysis. These dishes were used on an average of 150–200 times a year by several different chemists over Bunsen and Fisher burners and in muffles at temperatures up to 900°C.

For cleansing, the dishes were rinsed with warm water and dilute HCl, and were finally rubbed with a wet, well-soaped (ordinary laundry soap) swab of cheese cloth dipped in sea sand. In some cases it was necessary to use potassium bisulfate fusion to clean them.

Twenty-five dishes of 50 ml. capacity with an average weight of 24.2 grams, lost approximately 0.5 gram in 27–29 years; seven 100 ml. dishes, with an average weight of 47.5 grams, lost approximately 0.6 gram during 29 years of use. These results represent an annual loss of approximately 18 mg. of platinum per dish (irrespective of size) and at the price of platinum (approximately \$1.75 per gram) the loss amounts to three cents per dish per year. This cost is probably less than that of any other type of dish. While the unit expenditure for platinum dishes is high, the upkeep is low when the convenience of this container is considered.

\* By A. E. MIX (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

## BOOK REVIEW

*The Microscope and Its Use.* By FRANK J. MUNOZ, Technical Microscope Consultant, in Collaboration with Dr. Harry A. Charipper, Prof. of Biology, New York University. Chemical Publishing Co., Inc., Brooklyn, N. Y. Price, \$2.50.

The opening chapter of this book introduces the reader to the early development of the microscope. Chapter II describes monocular and binocular types of biological microscopes, and it is followed by a chapter on suitable illumination and various types of microscope lamps. A chapter is also included on the use of the microtome in microscopical work. Chapters VI–VIII describe the stereoscopic, metallurgical, and polarizing microscopes and their accessories. Copious illustrations, many of them from technical catalogues, a glossary, selected bibliography, and indices complete the descriptive material.

The aim of the authors has been to present the material in such non-technical language as to serve as a practical guide "to aid technicians and students in the use of the instrument." With this idea in mind, a handbook has been produced which will make a greater appeal to the beginner than to the specialist. The few typographical errors noted and omission of dates in the bibliography can be rectified in a later edition.—GEORGE L. KEENAN.

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